

TITANIUM DIOXIDE NANOPARTICLE INTEGRATED CONCRETE: AN
ASSESSMENT OF NANOPARTICLE RELEASE WHEN EXPOSED TO UV
RADIATION AND WET WEATHER CONDITIONS

by

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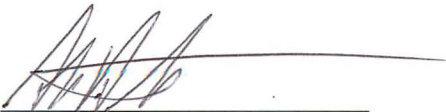
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DEDICATION

I dedicate this thesis to my wife and kids, mother, father, mother-in-law, and father-in-law, whose support greatly influenced my ability to achieve this accomplishment. Their encouragement, patience, and understanding facilitated my completion of this thesis.

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A handwritten signature in black ink, appearing to read 'D. J. Harrison', written over a horizontal line.

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April 1st, 2016

ABSTRACT

Titanium dioxide nanoparticle integrated concrete: An assessment of nanoparticle release when exposed to UV radiation and wet weather conditions:

CPT Daniel Harrison, Master of Science in Public Health, 2016

Thesis directed by: Lieutenant Colonel Christopher A. Gellasch, Assistant Professor, Department of Preventive Medicine and Biostatistics, Occupational and Environmental Health Sciences Division.

Nanomaterials have become increasingly utilized in new commercial products and technologies without a complete understanding of their impact on the environment or human health. One such application is the integration of titanium dioxide nanoparticles (nano-TiO₂) in concrete for exploitation of its photo-catalytic properties as a self-cleaning construction material. There is a lack of knowledge regarding how nanomaterials utilized in this manner may release from concrete during normal weathering and potentially affect the environment and human health. This study investigated the release of nano-TiO₂ integrated with concrete substrate when photocatalytically activated and immersed in acidic, neutral, and alkaline solutions that simulated rainfall in an effort to quantify detectable release of nano-TiO₂. This release-testing model simulates an annual amount (360 days) of ultraviolet (UV) exposure and an annual amount (30 rain events) of acidic

rain exposure. Effluent from each rinse was analyzed by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) for total titanium concentrations. Single Particle Inductively Coupled Plasma – Mass Spectrometry (SP-ICP-MS), a relatively new method of measuring nanoparticles in an aqueous solution, was performed to confirm and provide evidence of TiO₂ nanoparticle release. Results from this study indicated that there was a significant difference ($p = 0.000$) in the amount of titanium released in UV exposed samples when compared to UV unexposed samples. In contrast, there was no significant difference in the amount of titanium released attributed to the different pH conditions in both UV exposed ($p = 0.024$) and UV unexposed ($p = 0.126$) samples. This research provides evidence that titanium dioxide nanoparticle release in concrete applications is dependent on exposure to UV radiation and may not be associated with different pH conditions of wet weathering. This finding indicates that titanium dioxide nanoparticle integrated concrete can be classified as a source for titanium dioxide nanoparticle exposures when weathered under UV conditions, potentially affecting environmental and human health.

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CHAPTER 1: INTRODUCTION

Nanomaterials have advanced our daily lives by dramatically improving the products we use and the services on which we depend. Since their development, primarily due to the tunneling electron microscope, nanomaterials have become more widely used, ranging from the electronics we use to the buildings we work in. Their introduction in commercial products has been heralded as revolutionary, but not without concern. As with any material, there is a possibility for short and long-term adverse health impacts from exposure. Nanomaterials present a unique concern because of their small size and quantum properties. Compounding this, they have been widely incorporated into products without a complete understanding of their effects on human and ecological health.

NATURAL AND ENGINEERED NANOPARTICLES

The definition of a nanomaterial is fairly ambiguous, which makes it difficult to adequately identify and regulate a nanomaterial. In 2011, the European Union defined a nanomaterial as “a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm”(7). The United States Environmental Protection Agency (EPA) and other organizations do not clearly define what is considered a nanomaterial; however, the EPA describes nanomaterials in several documents as matter “between approximately 1 and 100 nanometers”(45). Generally, scientists classify nanomaterials as any natural or

engineered material with length between 1 and 100 nm in one dimension and exhibit properties unique to their dimensions (35).

The difference between nanomaterials and bulk or macro-scale materials is not just their size, but also the properties they exhibit. Nanomaterials often exhibit properties uniquely different from their macro-form, or significantly magnify the properties found in their macro-form. This is primarily due to their larger reactive surface area, but also because of the quantum interactions only possible at the nano-scale (46). Quantum reactions may change aspects of a material such as its fluorescence, conductivity, or chemical reactivity. For example, bulk aluminum is a stable material, but very combustible on the nano-scale. To demonstrate the surface area of a nanomaterial, consider a cube that is one centimeter in all dimensions. This cube would have a surface area of 6 square centimeters. Break that cube into cubes that are one nanometer in all dimensions, these cubes combined would have a surface area of 60 million square centimeters. That is a surface area seven orders greater than the macro-sized cube. This increased surface area provides far greater surface area on the atomic level for physical and chemical reactions to occur.

Natural nanomaterials have been in our environment since the beginning of time. Natural nanomaterials are formed in ash from volcanic eruptions, as a result of surface weathering, and by other natural methods. Only recently has the development of engineered nanomaterials been possible. Engineered nanomaterials are nanomaterials that are made either from larger bulk material or pieced together atom-by-atom. These non-natural materials can have unique properties not normally found in the environment and can pose a new threat to human and ecological health. Engineered nanomaterials are

of particular concern, not only because they have novel properties, but also because they exist on the cellular scale and have a potential to interrupt or alter normal cellular function (11). Carbon nanotubes, an engineered carbonaceous nanomaterial, are used in a myriad of products ranging from water treatment to tennis rackets. Carbon nanotubes have been fairly well researched since their development in the mid 1990's. Their fibrous structure acts to strengthen products making them extremely durable. Since 2004, animal studies have shown that carbon nanotubes can cause pulmonary granulomas (21), interstitial fibrosis (25), pleural fibrosis (41), and other systemic reactions (30). Silver nanoparticles, commonly used as an antimicrobial additive in clothing, are suspected to have adverse impacts on environmental ecology as well as microbial interactions within the human body. A review of research suggests that silver nanoparticles interrupt cellular function through the creation of reactive oxygen species (ROS) as well as interactions due to direct contact between released silver ions and cellular constituents (26). These interactions include cell membrane separation from the cytoplasm, DNA replication disruption, as well as direct damage to cellular lipids and DNA. Engineered titanium dioxide nanoparticles, although not well researched, are suspected of having carcinogenic and respiratory effects on human health (14).

Exposure to engineered nanomaterials can occur throughout all stages of its lifecycle. In the manufacture of nanomaterial-containing products and the synthesis of engineered nanomaterials, occupational exposures commonly occur via inhalation of aerosolized nanoparticles. A study looking at the potential for exposure to carbon-based nanomaterials in a laboratory setting identified increased amounts of nanomaterials in the air due to regular laboratory procedures (17). In a retrospective study by Boffetta *et al.*

(4), workers in a European titanium dioxide factory were exposed to an annual average of 0.1 to 1 mg/m³ TiO₂ dust. The highest annual average exposure, at an individual occupation process within the factory, was as much as 5 mg/m³. In a study performed by Koivisto *et al.* (20), workers in a nano-titanium dioxide pigment packing plant were exposed to average air concentrations of 225 to 700 µg/m³. Exposure to nanomaterials has also been shown in the normal use of products containing nanomaterials. In a study looking at the release of nanoparticles incorporated as fillers in dental composites, investigators found that nanoparticles in the range of 38 and 70 nm were released during abrasive procedures into the breathing zones of both the patient and dental personnel (47). In a study looking at the release of nano-silver used in food containers, it was found that nano-silver in both the dissolved and particle form were released (6). Nanomaterials can also become a source of exposure from the end-of-life or disposal of a nanomaterial containing product. Hennebert *et al.* (13), evaluated leachate from 25 different solid industrial and municipal waste facilities for the presence of nanoparticles and found, through TEM-EDS analysis, TiO₂, organic polymer, and particles containing Sr, La, Ce, and Nd. Additionally, various nanomaterials, such as nano-silver, titanium dioxide, and zero-valent iron were reported in wastewater streams.

TITANIUM DIOXIDE NANOPARTICLES

Titanium dioxide forms several metal oxide minerals, such as rutile, anatase, and brookite. Rutile is the most common naturally occurring titanium dioxide mineral and can be naturally or synthetically produced. It is primarily referred to as “pigment-grade” titanium dioxide as it is used as a whitening pigment in paints. Anatase is a less naturally common titanium dioxide mineral. It is mostly produced synthetically by hydrolysis of

titanium tetrachloride. Anatase titanium dioxide exhibits stronger photocatalytic properties, which makes it the preferred form for titanium dioxide nanoparticle production. Brookite is the least common natural form of titanium dioxide and is generally not used for any particular purpose.

NOVEL PROPERTIES OF TITANIUM DIOXIDE NANOPARTICLES

Titanium dioxide nanoparticles have been increasingly used in products since 1972 when Dr. Akira Fujishima of Kanagawa University and Dr. Kenichi Honda of the University of Tokyo published their research describing the Honda-Fujishima effect, which describes titanium dioxide nanoparticle's photocatalytic and superhydrophilic properties (8). These properties are the basis for titanium dioxide's "self-cleaning" attribute. Photocatalysis is the initiation and acceleration of a chemical process through the excitation of a molecule by photonic absorption (Figure 1). In titanium dioxide nanoparticles, the significantly larger surface area of the material magnifies this property.

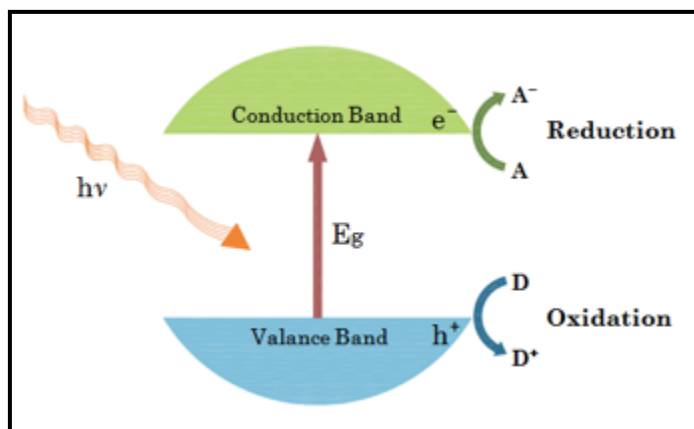


Figure 1: Illustration of TiO₂ photoactivation (1)

UV photons interact with the electrons in the titanium atom's valence shell causing them to move to a higher energy conduction band and leaving an electron hole in the valence band (43). The excited electrons interact with O_2 in the atmosphere forming super oxides (O_2^-). The electron holes interact with moisture in the air forming hydroxyl radicals (OH^\bullet). These ROS interact with organic matter, reducing them to CO_2 and H_2O . The electron hole formations also contribute to titanium dioxide nanoparticles' superhydrophilic property (Figure 2).

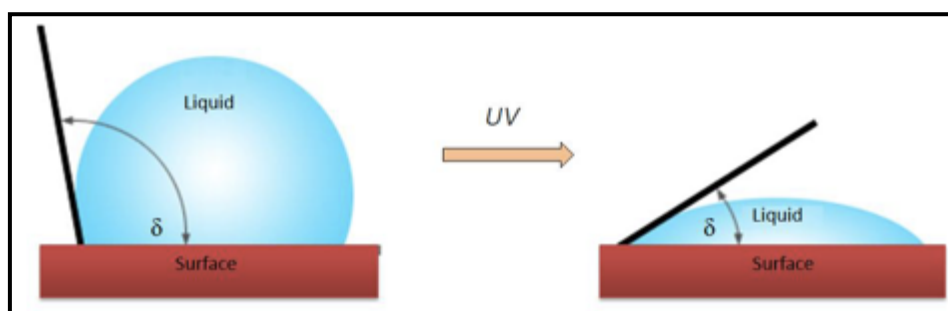


Figure 2: Illustration of superhydrophilic property showing the contact angle on non-photoactivated titanium dioxide nanoparticle surfaces compared to the contact angle on photoactivated titanium dioxide nanoparticle surfaces (1)

The electron holes act to weaken the titanium atom's bonds with oxygen, releasing oxygen from the titanium dioxide molecule. This produces surface vacancies in which water molecules bond, reducing water tension and contact angle. When the surface contact angle is reduced to $0^\circ \pm 1^\circ$, water droplets spread out creating a thin uniform layer. This layer is able to "self-clean" by wedging between contaminants and the titanium dioxide nanoparticle layer, picking up the contaminant and then running off the surface.

There are many products available that take advantage of titanium dioxide's properties. Sunscreens containing titanium dioxide nanoparticles block the harmful

effect of UV radiation by absorbing UV photons in photocatalysis. It also allows the sunscreen to be applied in a clear coat versus a thick white coat typically associated with sunscreens containing macro-rutile titanium dioxide or zinc oxide. Titanium dioxide nanoparticles have also been incorporated in concrete, roofing tiles, and pavement. These applications exploit titanium dioxide's self-cleaning property to prevent the growth of organic material and abate air pollutants like volatile organic chemicals and nitrous oxides. Titanium dioxide nanoparticles are even incorporated in glass as an anti-fogging agent, taking advantage of their superhydrophilic properties.

EXPOSURE AND HEALTH IMPACTS

As a result of nano-titanium dioxide's ubiquity in consumer and commercial products, there is now a greater potential for exposure to titanium dioxide nanoparticles than what was previously experienced. Exposures to titanium dioxide range from occupational exposures in factory settings, to exposures among the general population in the products we use, to exposures related to the disposal of products containing titanium dioxide nanoparticles (29,38-40). Animal *in vivo*, *in vitro*, and human epidemiological studies all claim and reject associations between titanium dioxide nanoparticles and adverse health effects (14).

Health concerns associated with titanium dioxide nanoparticles are primarily concerned with the reactive oxygen species generated by TiO₂ nanoparticle's photocatalytic reactions (48). Reactive Oxygen Species are generally responsible for causing cellular damage or apoptosis via oxidative stress. This is beneficial when trying to maintain a surface free of organic material; however, it can potentially be harmful to human and ecological health. Other concerns include the physical effects titanium

dioxide nanoparticles may have when inhaled, such as obstructive disorders and fibrosis. Several animal and epidemiological studies have been performed investigating inhalation, dermal, and ingestion exposure routes of TiO₂ nanoparticles (14). Despite the evidence provided by these studies, there is conflicting scientific evidence that makes it difficult to accurately conclude what adverse human health impacts can be attributed to TiO₂ nanoparticles. The American Conference of Governmental Industrial Hygienists (ACGIH) classifies titanium dioxide, regardless of size, as an A4 carcinogen: “not classifiable as a human carcinogen.” The International Agency for Research on Cancer (IARC) lists titanium dioxide, regardless of size, as a Group 2B carcinogen: “possibly carcinogenic to humans.” The National Institute of Occupational Safety and Health (NIOSH) is the only organization that specifically recognizes titanium dioxide nanoparticles as a potential human carcinogen.

Inhalation Exposures

In an acute inhalation study performed by Warheit *et al.* (49), rats were intratracheally exposed to 1 or 5 mg/kg of two different sizes of rutile titanium dioxide nanoparticles or one size of Degussa P25 titanium dioxide nanoparticles. Degussa P25 titanium dioxide nanoparticles is a commonly used commercial product that is 70% anatase TiO₂ with crystalline sizes of approximately 20nm. The rats were evaluated at 24 hours, 1 week, 1-month, and 3-month time points for bronchoalveolar lavage fluid inflammatory markers, cell proliferation, and histopathology. Exposure to the P25 titanium dioxide nanoparticles produced pulmonary inflammatory responses, cytotoxicity, and adverse lung tissue effects, while the rutile nanoparticles produced limited transient inflammation. The authors concluded that the response from P25

exposure might be due to the differences in crystalline structure, pH of the particle, or surface chemical reactivity.

In a sub-chronic inhalation study performed by Oberdörster *et al.* (11), rats were exposed to two different sized anatase titanium dioxide particles, one nanoparticle size (~20nm) and one fine particle size (~250nm). The study was conducted for 12 weeks. The authors found significant deposition of nanoparticles in the lower respiratory tract causing high inflammatory response, type II cell proliferation, pore occlusion, interstitial fibrosis, and impairment of alveolar macrophage function. These effects were not observed with the larger fine-grade of titanium dioxide.

In several chronic inhalation studies, mostly from the mid to late 1980s, there was no correlation found between titanium dioxide exposure and cancer formation; however, it is important to note that it is unclear what form of titanium dioxide these studies used (22,44,51). During the 1980s, macro-sized titanium dioxide particles were commonly used in paints and plastics as a whitening agent. Titanium dioxide nanoparticles were not commonly used or understood until the mid 1990s. It is likely that these studies used macro-scale rutile titanium dioxide versus nano-scale anatase titanium dioxide in their studies.

Human epidemiological studies of titanium dioxide focus on occupational exposures from TiO₂ factories ranging from the mid-1980s to the early 2000s (4,5,38). All the reviewed studies indicate that there is no carcinogenic correlation with TiO₂ exposure and limited pulmonary inflammation and fibrotic response. Again, it is unclear what size and structure of titanium dioxide these exposures were attributed to.

Dermal Exposures

Dermal exposure studies conducted in the past two decades include studies on human skin and animal skin and primarily look at titanium dioxide's ability to penetrate the stratum corneum, the layer of skin that protects the body from exterior exposures. These studies differ in that the *in vitro* studies show no penetration of the stratum corneum, whereas the *in vivo* animal studies show penetration into the lower layers of skin, but not the dermis (9,28,40). Of the few *in vivo* human studies, all show that there is no penetration of the stratum corneum (29,42). Dermal exposures are of particular interest to consumers as titanium dioxide is increasingly used in sunscreens and cosmetics as a UV blocking agent.

In an acute *in vivo* human dermal exposure study performed by Schulz *et al.* (42), volunteers applied 45 mg of four different test emulsions of titanium dioxide nanoparticles on their forearm for 6 hours. From these volunteers' forearms, 2 mm punch biopsies were taken and analyzed under tunneling electron microscopy (TEM) and light microscopy. Based on the analyses performed, the authors concluded that the surface characteristics, structure, and particle size of titanium dioxide nanoparticles had no effect on the absorption of the nanomaterial past the stratum corneum.

In an acute *in vivo* animal dermal exposure study performed by Menzel *et al.* (28), researchers applied four commercially available formulations of titanium dioxide nanoparticle containing sunscreens on live pig skin. Punch biopsy samples were taken 8, 24, and 48 hours later and analyzed for titanium dioxide nanoparticles. The researchers found penetration through the stratum corneum via intercellular spaces into the stratum granulosum within 8 hours of exposure.

In several *in vitro* human and animal studies, investigators found that there was no evidence of penetration by titanium dioxide nanoparticles in the stratum corneum (9,40). Testing on compromised animal skin has also shown no penetration of titanium dioxide nanoparticles in the stratum corneum (29). This contradiction in findings leaves the route of dermal exposure uncharacterized in regards to titanium dioxide nanoparticles.

Gastrointestinal Exposures

There is a sufficient amount of current research investigating the effects and distribution of titanium dioxide nanoparticles from an ingestion exposure route, as TiO₂ nanoparticles are finding an increasing presence in pharmaceuticals, cosmetics, food packaging, and agriculture. *In vivo* and *in vitro* animal studies have shown that TiO₂ nanoparticles pass through the gastrointestinal tract into the blood system and distribute to a variety of tissues, primarily the liver, kidneys, spleen, and lungs (14).

In a recent *in vivo* human study conducted by Pele *et al.* (39), seven volunteers were orally administered two 50 mg capsules of food-grade TiO₂, approximately 250 nm in size. Nine blood samples were then taken from baseline through 10 hours post-exposure and analyzed by dark field spectrometry and induced coupled plasma mass spectrometry for total titanium blood concentration. The authors concluded that the TiO₂ particles were distally and proximally distributed from the gut, but that further research needs to be conducted investigating the amount of particles and extent of distribution.

In an *in vivo* animal study conducted by Wang *et al.* (48), rats were orally administered 5g/kg of TiO₂ nanoparticle suspension by syringe into the gastrointestinal tract and sacrificed after two weeks. Serum, heart, liver, spleen, kidneys, lung, brain and testicle samples were excised and analyzed for titanium content by inductively coupled

plasma mass spectrometry. The authors concluded that there was distribution to different tissues of the rat and observed hepatic injury, myocardial injury, and nephrotoxic injury. There were no pathological changes in the spleen, heart, testicles, or lungs, but that the TiO₂ particles of various sizes were retained in the lung, liver, spleen, and kidneys. Alternatively, in a combination *in vitro/in vivo* study performed by MacNicoll *et al.* (24), rats and epithelial tissues were exposed to TiO₂ nanoparticle solutions. It was found that there was no translocation of nanoparticles past the gastrointestinal epithelium and that a majority of the TiO₂ nanoparticles was eliminated in the feces.

The epidemiological and animal research that has been performed demonstrates that there is a potential for titanium dioxide nanoparticles to impact short and long-term human health. This is significant in determining the future use of titanium dioxide nanoparticles in consumer and commercial products. Additionally, it identifies a need to determine the exposure route in which nano-titanium dioxide may impact a population.

REGULATORY EXPOSURE LIMITS

Regulatory limits pertaining to titanium dioxide are based on animal and human epidemiological studies performed. Limits have been established primarily based on the adverse health impacts evident in the animal studies, but are not significantly established because of the lack of evidence supporting carcinogenicity in human epidemiologic studies (51). The only existing limits established for titanium dioxide nanoparticles are those recommended by the National Institute of Safety and Health (NIOSH), which pertain to occupational inhalation exposures, the primary mode of exposure for titanium dioxide. NIOSH's recommended exposure limit (REL) for fine titanium dioxide (0.1-3 µm) and ultra-fine titanium dioxide (0.1 – 100 nm) is 2.4 mg/m³ and 0.3 mg/m³ with a 10-

hour time weighted average (TWA), respectively. The Occupational Safety and Health Administration (OSHA) bases their permissible exposure limit (PEL) on NIOSH's recommendation. The OSHA PEL is currently 15 mg/m³ with an 8-hour TWA (Figure 3). There are currently no limits established in the Safe Drinking Water Act or Clean Water Act for titanium dioxide of either particle size.

TiO ₂		
Agency	Single-shift TWA (mg/m ³)	Comments
NIOSH [2002]	--	Potential human carcinogen
OSHA	15	Total
ACGIH [2001a, 2001b, 2005, 2009]	10	Category A4 (not classifiable as a human carcinogen); TiO ₂ is under study by ACGIH [ACGIH 2009]. Inhalable fraction except for ultrafine particles; suspected carcinogen (MAK Category 3A)
MAK [DFG 2000, 2008]	--	
Abbreviations: ACGIH = American Conference of Governmental Industrial Hygienists; MAK = Federal Republic of Germany Maximum Concentration Values in the Workplace; NIOSH = National Institute for Occupational Safety and Health; OSHA = Occupational Safety and Health Administration		

Figure 3: Occupational exposure limits and guidelines for TiO₂ (34)

MEASUREMENT OF TITANIUM DIOXIDE NANOPARTICLES

Measurement of titanium dioxide nanoparticles is difficult due to their size, properties, and given matrix (23). Different analytical techniques have been developed to measure nanoparticles and these techniques differ based on the nanoparticle measured.

These techniques often combine several analytical methods to achieve the desired measurement. In the case of titanium dioxide nanoparticles in an aqueous solution, a common technique is filtration, ultra centrifugation, and analysis by ICP-MS.

Quantitative measurement of titanium dioxide nanoparticles in solution, is most commonly analyzed by either Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) or Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) (23). These analytical methods provide total titanium and other elemental concentrations. Typically, solutions must undergo a form of particle separation, such as centrifugation or fractionation, and acid digestion prior to analysis. Advantages to this method of analysis are that most labs have these instruments, they are relatively simple to operate, and can detect concentrations as low as parts per billion. Titanium Dioxide Nanoparticles have been successfully detected in studies evaluating both surface water and wastewater treatment plant effluent using these analytical methods (16,18,19,36,50).

Two relatively novel techniques, Field Flow Fractionation (FFF) and Single Particle – Inductively Coupled Plasma – Mass Spectrometry (SP-ICP-MS), are being used to characterize the particle size of nanomaterials (23). Field flow fractionation uses a laminar flow channel and a cellulose membrane with a perpendicular cross flow to separate out different sized particles in a solution. As a sample solution is pumped through the inlet into the flow channel, a parabolic flow profile forms. This allows a cross flow to separate out particles by size. When coupled with an ICP-MS, this gives researchers a signal-intensity vs. time curve that shows the presence of different sized particles. Single particle ICP-MS involves the measurement of nanoparticles one particle at a time. Software developments for the ICP-MS and methods that slow down the

inflow of sample solution allow the ICP-MS to analyze nanoparticles individually giving a size distribution for the sample analyzed. The method takes a great level of experience, as a flow that is too fast will result in multiple particles being analyzed at once.

Combining these two techniques provides potential for more sensitive and accurate particle measurement. Although these techniques provide a means of measuring nanoparticles, it takes a highly trained professional in these techniques to make accurate nanoparticle measurements.

Qualitative analysis of nanomaterials has primarily depended on the use of Scanning Electron Microscopy (SEM) or Tunneling Electron Microscopy (TEM) to evaluate nanoparticles in different media for size, distribution, and shape of nanoparticles (23). These analytical techniques help to inform the quantitative analysis of nanoparticles and determine appropriate combinations of analytical methods.

DURABILITY STUDIES

There have been several studies performed examining the durability of photocatalytic nanomaterials on different substrates, undoubtedly due to the need for validation and improvement of commercial applications. The majority of these studies investigate surface applications of titanium dioxide mortars or paints and often neglect to look at the durability of the product in the sense of it being retained in its applied form (2,10,12,27,37). These studies are primarily concerned with the durability of the product as it pertains to titanium dioxide's photocatalytic performance. Any qualitative analysis of titanium dioxide conducted in these studies only looked at durability of the coating under SEM imaging or weighing/thickness measurements. There was only one study found that involved titanium dioxide nanoparticles integrated in concrete; however, it did

not measure titanium dioxide nanoparticles, but measured the impact that titanium dioxide nanoparticles had on the concrete's hydration, compressive strength, setting time, and surface microhardness. (3).

RESEARCH PURPOSE

Titanium dioxide nanoparticles are increasingly integrated with concrete to enhance construction and infrastructure applications without a complete understanding of the product's life cycle and impact on the environment and human health. It can be assumed that normal weather conditions can cause the release of titanium dioxide nanoparticles from their concrete substrates and into the environment. Depending on the fate and transport of titanium dioxide nanoparticles, there is a potential for the application to be an exposure source. Figure 4 shows a conceptual model for the exposure of a population to titanium dioxide nanoparticles. In this model, nano-titanium dioxide integrated applications are compromised by acidic precipitation and released into the surface water runoff. Carried by the surface water, the nanoparticles may reach a drinking water system where it can then be distributed and consumed by the general population.

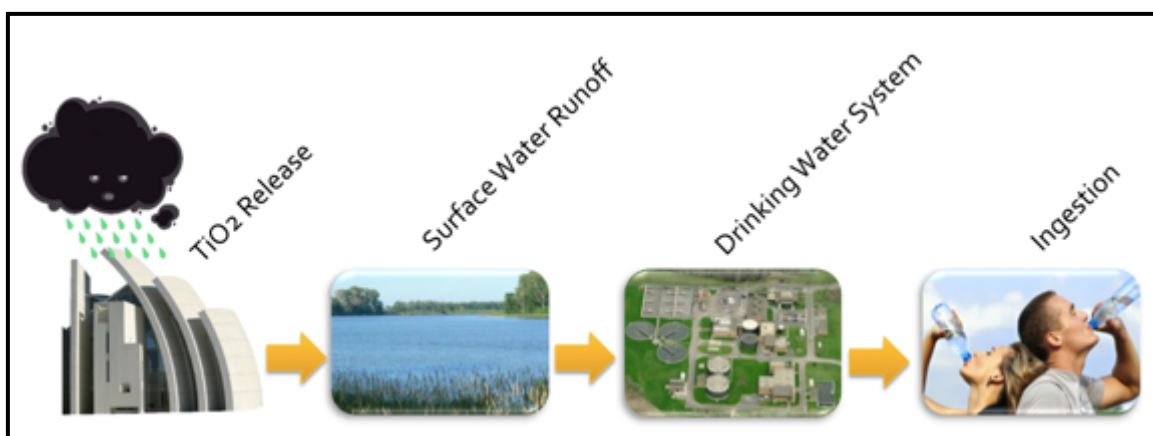


Figure 4: Conceptual model for exposure to titanium dioxide nanoparticles

The purpose of this study is to investigate the release of nano-TiO₂ integrated with concrete substrate when photocatalytically activated and immersed in acidic, neutral, and alkaline solutions that simulated rainfall in an effort to quantify detectable release of nano-TiO₂. This research will investigate the potential for this application as a source of exposure to titanium dioxide nanoparticles.

Hypotheses

1. TiO₂ nanoparticles wet weathered in acidic solution will release from concrete substrate in larger concentrations than TiO₂ nanoparticles wet weathered in neutral and alkaline solutions.
2. Photoactivated TiO₂ nanoparticles will release from concrete substrate in larger concentrations than non-photoactivated TiO₂ nanoparticles.
3. Photoactivated/Acidic Wet Weathered TiO₂ nanoparticles will release from concrete substrate in larger concentrations than non-photoactivated/standard, neutral and alkaline wet weathered TiO₂ nanoparticles.

Specific Aims

1. Quantitatively analyze the total concentration of titanium, magnesium, and calcium released by ICP-MS and ICP-AES methods.
2. Quantitatively analyze size distribution of titanium dioxide nanoparticles using SP-ICP-MS.
3. Qualitatively analyze samples for nanoparticle release using SEM imaging.

CHAPTER 2: METHODS

The objective of the experiment conducted was to measure the release of titanium dioxide nanoparticles integrated with a concrete substrate weathered under laboratory simulated ultraviolet and acidic precipitation conditions. Samples were prepared, then weathered using an accelerated ultraviolet weathering instrument and subsequently immersed in different pH solutions for an equivalent of one year's exposure. Release was quantitatively measured using inductively coupled plasma – mass spectrometry and single particle inductively coupled plasma – mass spectrometry. Scanning electron microscopy was used to qualitatively examine titanium dioxide nanoparticle morphological differences between un-weathered and weathered samples.

CONCRETE TEST PUCK PREPARATION

Concrete pucks integrated with titanium dioxide nanoparticles were produced by the United States Army Corps of Engineers – Engineer Research and Development Center (USACE – ERDC) Concrete and Materials Branch (CMB) personnel in accordance with mixture proportions in American Society for Testing and Materials (ASTM) standard C109. A standard siliceous aggregate was used as recommended by the standard. Baseline samples used conventional ASTM C150 Type I/II Portland cement. Test materials replaced the cement fraction with TxActive™ titanium dioxide material. The nano-titanium dioxide integrated concrete was produced as 101.5 mm diameter by 203 mm tall cylinders and moist cured in a fog room for seven days followed by drying. Cylinders were cut into eight 17.5 mm tall cylinders in which sixteen 12.7 mm diameter pucks were bored out (Figure 5). Test pucks were 28 days old at the beginning of testing and as a result, in their fully reacted state.



Figure 5: Image of titanium dioxide integrated concrete test puck (17.5 mm tall by 12.7 mm diameter) after testing

CONTAINER SELECTION

Borosilicate vials were selected for use as a container to hold the specimen and solution during the weatherization testing. Selection of an appropriate container was dependent on the highest, most consistent transmission of UV light through the container and the ability to immerse the test puck. Several materials were tested using an Ultraviolet – Visible (UV-VIS) light spectrometer to determine which had the most transmittance (Figure 6). These materials included plastic, borosilicate, and glass. Figure 6 shows the percent transmission versus the wavelength of UV light for borosilicate, glass, and plastic. The borosilicate spectra supported approximately 50% - 75% transmission through a UV light range of 300 nm – 400 nm, whereas the glass and plastic materials show 0% - 90% transmission through a UV light range of 300 nm – 400 nm.

The borosilicate scintillation vial was chosen based on its ability to hold the concrete test puck and its consistent moderately high transmittance of 75% throughout the 300 nm – 400 nm wavelengths. Although the plastic petri dish exhibited 60% - 90% transmittance throughout the 300 nm – 400 nm range, it was unable to hold enough solution to allow for the test puck to be immersed.

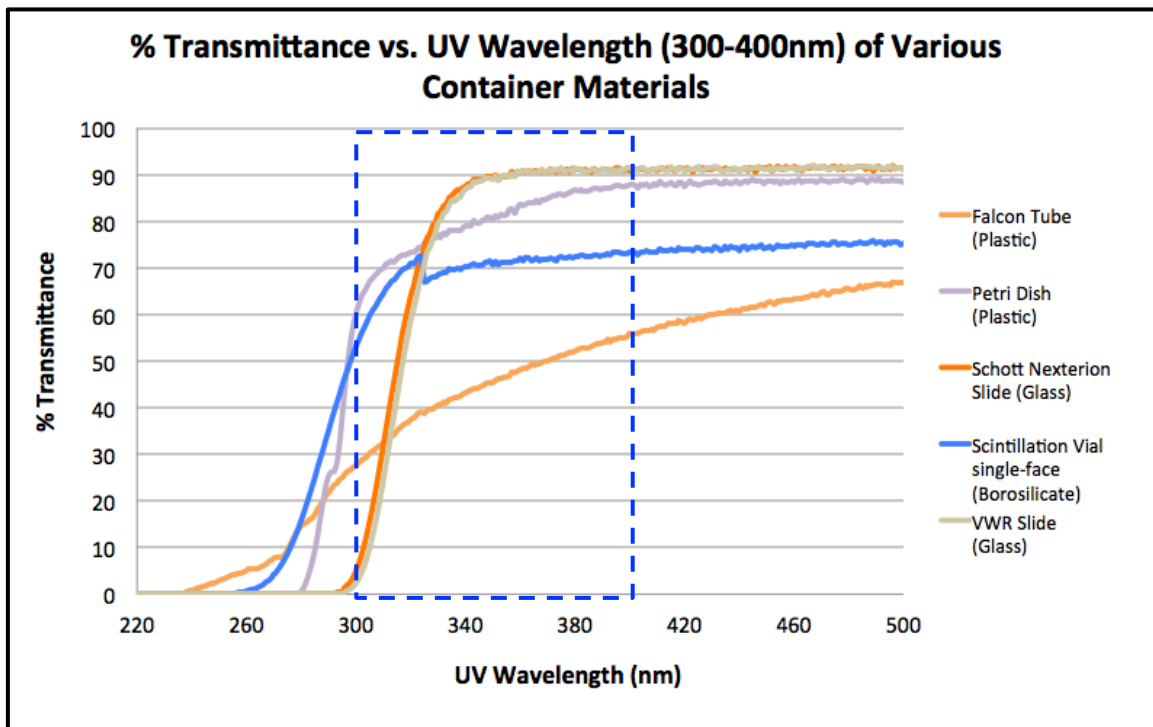


Figure 6: Percent transmittance of potential weatherization containers measured by ultraviolet-visible light spectrometry

SOLUTION PREPARATION

To test the effect of acid rain on the titanium dioxide nanoparticle integrated concrete, solutions of pH 4, pH 5.6, pH 7, and pH 9 were prepared. These solutions simulated a range of different rain conditions, allowing a comparison of differences between pH exposures. The pH 4 solution was used to simulate acid rainwater. The pH

5.6, 7, and 9 solutions represent (in order) natural rainwater, neutral pH rain, and very basic or alkaline rain. The solutions were prepared in one liter, brown, capped Nalgene™ bottles. After rinsing, the bottles were each filled with deionized water and then either NaOH or H₂SO₄ were added until the desired pH was attained within ± pH 0.1. pH was continuously measured using a digital pH meter (Figure 7). The solutions were used for wet weatherization within one hour of their formulation.



Figure 7: Solution Preparation

EQUILIBRATION DETERMINATION

The amount of time required for the pH 4 solution to reach equilibrium when exposed to the concrete test puck determined the exposure time for the immersive wet weatherization protocol. This amount of time was determined by exposing a single concrete test puck to 10 ml of pH 4 solution and then measuring the pH every minute

until no further change in pH was recorded. This was conducted once with one test puck. Colorimetric pH test strips were used to determine the pH during the exposure. It was determined that equilibrium at pH 11 was reached in 35 minutes, which is the exposure time used in the wet weatherization protocol.

PILOT STUDY

A pilot study was performed to validate the puck size, solution amount, and exposure time through obtaining measureable amounts of titanium by ICP-MS analysis. For this study, sixteen test pucks were exposed to ultraviolet light using the Atlas Suntest XLS+ accelerated weatherization instrument. Ultraviolet wavelength was set in accordance with ISO 4892-2/2013 to between 300 and 400 nm, irradiance to 60 ± 2 W/m², BST to $63 \pm 5^\circ\text{C}$, and CHT to $32 \pm 5^\circ\text{C}$. On/off cycles were set for one hour on and one hour off, which was an equivalent to 6 hours of sunlight and 6 hours of darkness. Six cycles were performed. Upon completion of each cycle, the test pucks were exposed to either pH 4, pH 5.6, pH 7, or pH 9 solutions for 35 minutes. The solution was then poured away from the test puck, acid digested with nitric acid, and analyzed by ICP-MS for total titanium concentration. Results from the pilot study validated the exposure method as measurable concentrations of total titanium were attained for each cycle performed.

EXPERIMENTAL SETUP

The experimental setup was designed to test the release of titanium dioxide nanoparticles when weathered under ultraviolet and immersive wet weather conditions. The testing method was adapted from the simulated weatherization protocol for carbon

nanotubes developed by NanoRelease (32). This protocol is patterned after the ISO 4892-2/2013 standard method for accelerated weatherization and is used by USACE-ERDC (15). Departures from the standard method include the size of the sample puck, exposure durations, and immersion exposure protocol. There were no methods found to base acidic immersion testing on for nanoparticle release.

Accelerated Ultraviolet Weatherization

Ultraviolet weatherization was performed using the Atlas Suntest XLS+ accelerated weatherization instrument. The Suntest XLS+ is a programmable bench-top chamber equipped with a 1700 W xenon lamp used to weather test samples with ultraviolet light. Ultraviolet wavelength, irradiance, chamber temperature, and black standard temperature can be set to provide a consistent environment for testing.

For the purposes of ultraviolet weatherization, 16 test concrete pucks were placed individually in borosilicate scintillation vials and then placed in a 4x4 matrix in the center of the Atlas XLS+ test chamber. The test pucks were subsequently exposed to continuous ultraviolet light for 24 hours to simulate an equivalent to 12 days ultraviolet light exposure (Figure 8).

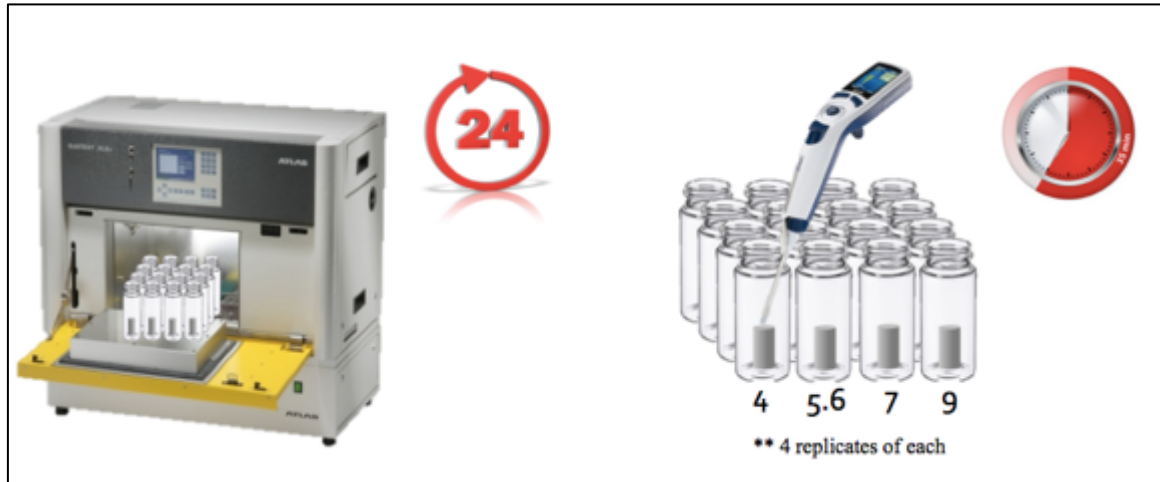


Figure 8: Illustration of UV and wet weather exposure protocol

Ultraviolet wavelength was set in accordance with ISO 4892-2/2013 to between 300 and 400 nm, irradiance to $60 \pm 2 \text{ W/m}^2$, BST to $63 \pm 5^\circ\text{C}$, and CHT to $32 \pm 5^\circ\text{C}$. Upon completion of the exposure program, each vial was immediately removed from the chamber and weathered according to the immersive wet weathering protocol. Following immersive wet weatherization, this procedure was repeated 30 times to simulate an annual equivalent of ultraviolet light exposure.

Immersive Wet Weatherization

Immersive wet weatherization was performed to assess the effect of acidic solution exposure on titanium dioxide nanoparticle release. There was no documented

method for performing this testing. Approximately one hour prior to exposure, solutions of pH 4, pH 5.6, pH 7, and pH 9 were prepared according to the solution preparation protocol previously described. Upon completion of the accelerated ultraviolet weatherization protocol, each borosilicate vial was filled with 10 ml of solution using a digital pipeter and exposed for 35 minutes. At 35 minutes, exposure was stopped by gently swirl-agitating each vial by hand and pouring the liquid contents into 10 ml BD Falcon™ round bottom polystyrene tubes. This procedure was repeated 30 times to simulate an annual exposure to acidic precipitation weathering. The average annual number of acidic precipitation events was determined from data obtained from the National Atmospheric Deposition Program which monitors rain events using precipitation monitoring stations across the United States (33). Table 1 provides an overview of the samples obtained by exposure group.

Table 1: Samples breakdown by UV and wet weather exposure

		# of Replicates	# of Exposures	Total Samples
UV Exposed	pH 4	4	30	120
	pH 5.6	4	30	120
	pH 7	4	30	120
	pH 9	4	30	120
UV Unexposed	pH 4	4	30	120
	pH 5.6	4	30	120
	pH 7	4	30	120
	pH 9	4	30	120
Totals		16	--	960

Mass Spectrometry Analysis

Inductively coupled – mass spectrometry (ICP-MS), Inductively coupled – Atomic Emission Spectrometry (ICP – AES), and single particle inductively coupled -

mass spectrometry (SP-ICP-MS) were used to quantitatively analyze effluent samples taken from the immersive wet weathering protocol. ICP – MS was used to analyze total titanium concentrations as it provided parts per billion concentration results and did not require the digestion of the sample with hydrofluoric acid (HF). HF acid is not only very hazardous to handle, but is considered a “dirty” acid, meaning that it contains trace amounts of other elements, titanium being one of them. Instead, Ultrapure nitric acid was used for the sample digestion. ICP – AES was used to analyze the samples for total calcium, which will be used to determine if a correlation exists between titanium release and calcium release. SP-ICP-MS, a fairly new analytical technique for the detection of nanoparticles, was used to detect the presence of nanoparticles in the sample solutions. SP-ICP-MS is a modified ICP-MS method, which slows down the flow of the analyte into the instrument allowing single particles within the solution to be analyzed one at a time. This provides an output of a particles size distribution within a sample. Upon completion of the immersive wet weathering, a 1 ml sample was taken by digital pipette, stored in a 1 ml amber glass sample vial, and refrigerated at 4 °C until analyzed by SP-ICP-MS. A 1 µL sample was taken by digital pipette and diluted up to 10 mL with diluted nitric acid in a 10 mL test tube. This sample was used for the ICP-AES analysis. The remainder of the sample solution was acid-digested with two drops of ultra pure nitric acid for ICP-MS analysis.

ICP-MS analysis was performed using a Perkin Elmer NexION 300D ICP- MS and United States Environmental Protection Agency (USEPA) method SW 846/6020 for total metals. ICP-AES analysis was performed using a Perkin Elmer Optima 8300DV ICP-AES and USEPA method SW 846/6010 for total metals. Sc, Y, Ge, Rh, In, Ho, and

Bi internal NIST traceable standards, used to correct for instrumental drift, were purchased from CPI International. SP-ICP-MS analysis was performed using Elan software operating in “nano” mode on the Perkin Elmer NexIon 300D with a dwell time of 100 microseconds and a total of 60 seconds per sample. Transport efficiency was determined using 60 nm NIST gold nanoparticles and the titanium calibration curve was generated using NIST calibration standards for dissolved titanium.

Scanning Electron Microscopy

Scanning electron microscopy images were taken to provide a qualitative comparison between test pucks prior to and after UV and wet weatherization exposures. Upon completion of the 30 exposures, the used test pucks were allowed to dry at room temperature and then imaged again using scanning electron microscopy for morphological analysis. Images were taken from a test puck prior to exposure. Images were also taken from a pH 4/UV exposed test puck and a pH 4/ UV unexposed test puck after their exposures.

DATA ANALYSIS

Data generated was reported from each analytical instrument in the form of an Excel data spreadsheet. Averages of replicates were calculated and organized by pH and UV exposure into a matrix for easier graphical analysis. Statistical Package for the Social Sciences (SPSS, Version 22) software was used to perform statistical analysis. One-way and two-way ANOVA statistical tests with a confidence level of 95% was used to determine the statistical difference in means between UV exposed and UV unexposed conditions, between pH conditions, and between UV exposed and UV unexposed conditions within pH conditions. A Spearman correlation test was used to determine the

relationship between calcium release concentrations and titanium release concentrations.

SEM images were used to provide information pertaining to the morphology of the test pucks.

CHAPTER 3: RESULTS AND ANALYSIS

Results from this experiment produced several types of data. Total titanium and calcium concentrations were obtained from ICP-MS and ICP-AES analysis, titanium dioxide nanoparticle size in solution was determined using SP-ICP-MS, and images of the concrete test puck prior to and after testing were obtained using SEM. Statistical analysis tables are available in appendix A and raw data is available on the attached compact disc.

SINGLE PARTICLE ICP-MS AND SEM RESULTS

Results gathered from the SP-ICP-MS indicate that titanium dioxide nanoparticles larger than 100 nm were detected in the sample solutions. Scanning electron microscopy images (Figures 9a – 9c) indicate that titanium dioxide nanoparticles had agglomerated during the concrete curing process to form larger, approximately 100 – 250 nm, titanium dioxide crystals.

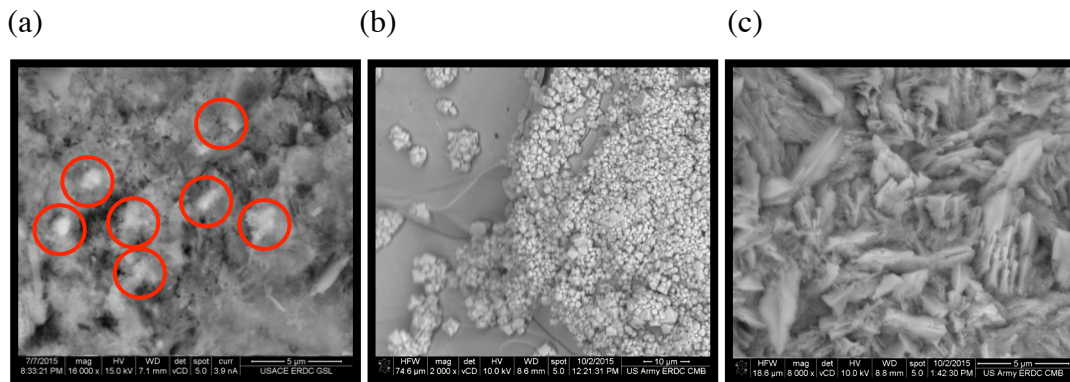


Figure 9: Scanning Electron microscopy images of titanium dioxide integrated puck (a) prior to ultraviolet and wet weather exposure, (b) after 30 exposures to pH 4 solution and no UV exposure, and (c) after 30 exposures to both UV and pH 4 solution. Image (a) and (c) are at a 5 μm scale. Image (b) is at a 10 μm scale.

The white areas in figure 9 (a) indicate the areas in which titanium dioxide nanoparticle agglomerates are embedded in the surface of the concrete test puck. Dark areas are the other compounds that make up concrete.

SEM images from test pucks exposed to both UV and pH 4 solution show some morphological changes that are of importance. First in figure 9 (b), after exposure to pH 4 solution and no exposure to UV, titanium dioxide nanoparticle agglomerates are clearly seen still embedded on the surface of the test puck. The percentage of these agglomerates released due to weathering is uncertain based on the image alone because an image at the same location was not taken prior to exposure. It might be concluded that the bare spots are areas where titanium dioxide nanoparticle agglomerates released during the weathering. Figure 9 (c) shows a very different result, most likely due to the UV exposure. The image shows calcium carbonate covering the surface of the test puck. It is likely that this is calcium carbonate that had released due to weathering and reprecipitated on the surface as a result of the heating/cooling and wetting/drying associated with the exposure tests. Again, it is difficult to determine the amount of titanium dioxide nanoparticle agglomerate released due to weathering. These results do however show what may be happening to the test pucks on the surface as a result of the weathering tests and may inform the numerical data obtained in the ICP-MS and ICP-AES analysis.

TITANIUM RESULTS

Titanium release was measured by ICP-MS, which analyzed effluent samples for total titanium concentrations, including both dissolved and particulate titanium. Results were averaged over the thirty exposures for each pH condition and UV condition for

comparison as depicted in figure 12. Results from the ICP-MS analysis indicated measureable concentrations of titanium greater than the instrument's reporting limit of 0.005 mg/L. As seen in figure 10, average total titanium concentrations ranged from 1.3 ppb to 2.3 ppb across the different exposure conditions. There is a visual difference in titanium release with a larger release in the UV exposed group as compared to the UV unexposed group. When comparing titanium release between the different pH conditions, there is not much of a visual difference between the pH conditions in both the UV exposed and the UV unexposed group.

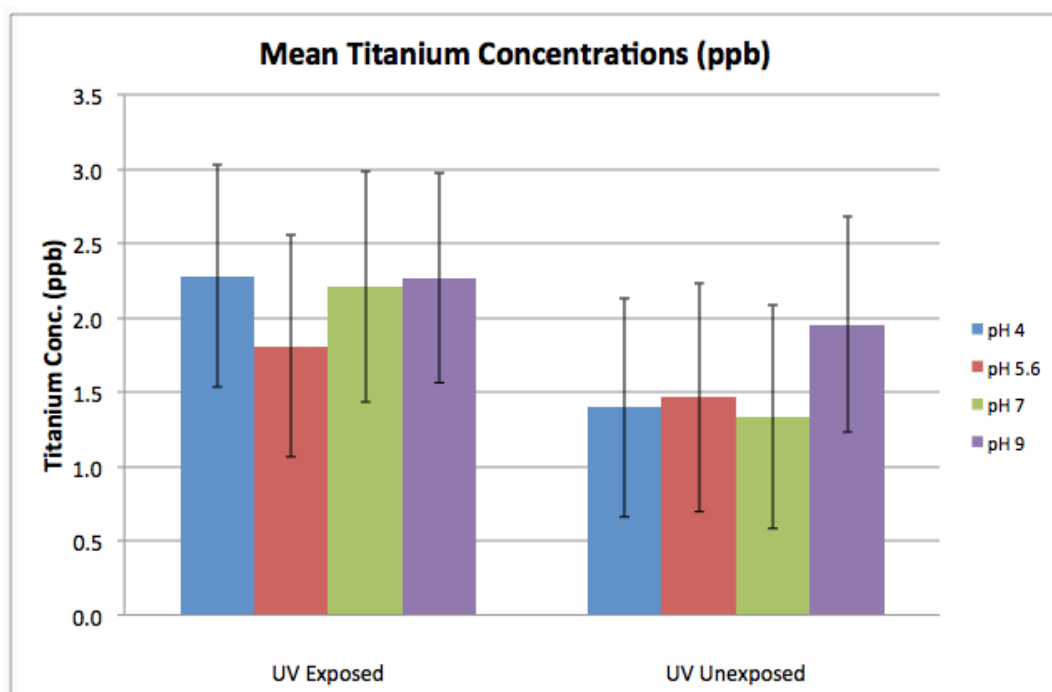


Figure 10: Mean titanium concentrations by UV exposure and pH exposure. Error bars represent the calculated standard deviation.

CALCIUM RESULTS

Calcium release was measured by ICP-AES, which analyzed effluent samples for total calcium concentrations, including both dissolved and particulate calcium. Calcium

results are depicted in figures 16 and 17. Total calcium measurements indicate parts-per-million (ppm) amounts of calcium with significantly greater amounts in the UV unexposed group compared to the UV exposed group. Additionally, there are two areas in the data that are of concern in making a correlation with the titanium data. First, in exposures one through three, there is an initially large concentration of calcium measured, which dramatically declines over the three exposures. This is similarly observed in both the UV exposed and UV unexposed calcium data (Figures 11-12). Second, in the UV exposed and UV unexposed datasets, there is a “jump” in calcium concentrations at exposure 16 in the UV exposed dataset and exposure 20 in the UV unexposed dataset (Figures 13-14). For the purpose of evaluating correlation in the data, the calcium data was split into four different groups (Table 2).

Table 2: Calcium Group Breakdown

Calcium Data Grouping	
Grouped Data	Exposures
UV Exposed Group 1	4 - 16
UV Exposed Group 2	19 - 30
UV Unexposed Group 1	10 - 20
UV Unexposed Group 2	21 - 30

These groupings were made based on their average concentration in relation to each other and within each UV exposure grouping. In “UV Group 1”, the average concentration is 1.97 ppm and the variance is 1.13 ppm. In “UV Group 2”, the average concentration is 4.84 ppm and the variance is 5.42 ppm. In “NUV Group 1”, the average concentration is 1.52 ppm and the variance is 2.48 ppm. In “NUV Group 2”, the average concentration is 12.23 ppm and the variance is 1.27 ppm. The large difference in average

concentrations between “UV Group 2” and “NUV Group 2” are potentially due to the effect of UV exposure and the formation of different concrete phases at those exposure points.

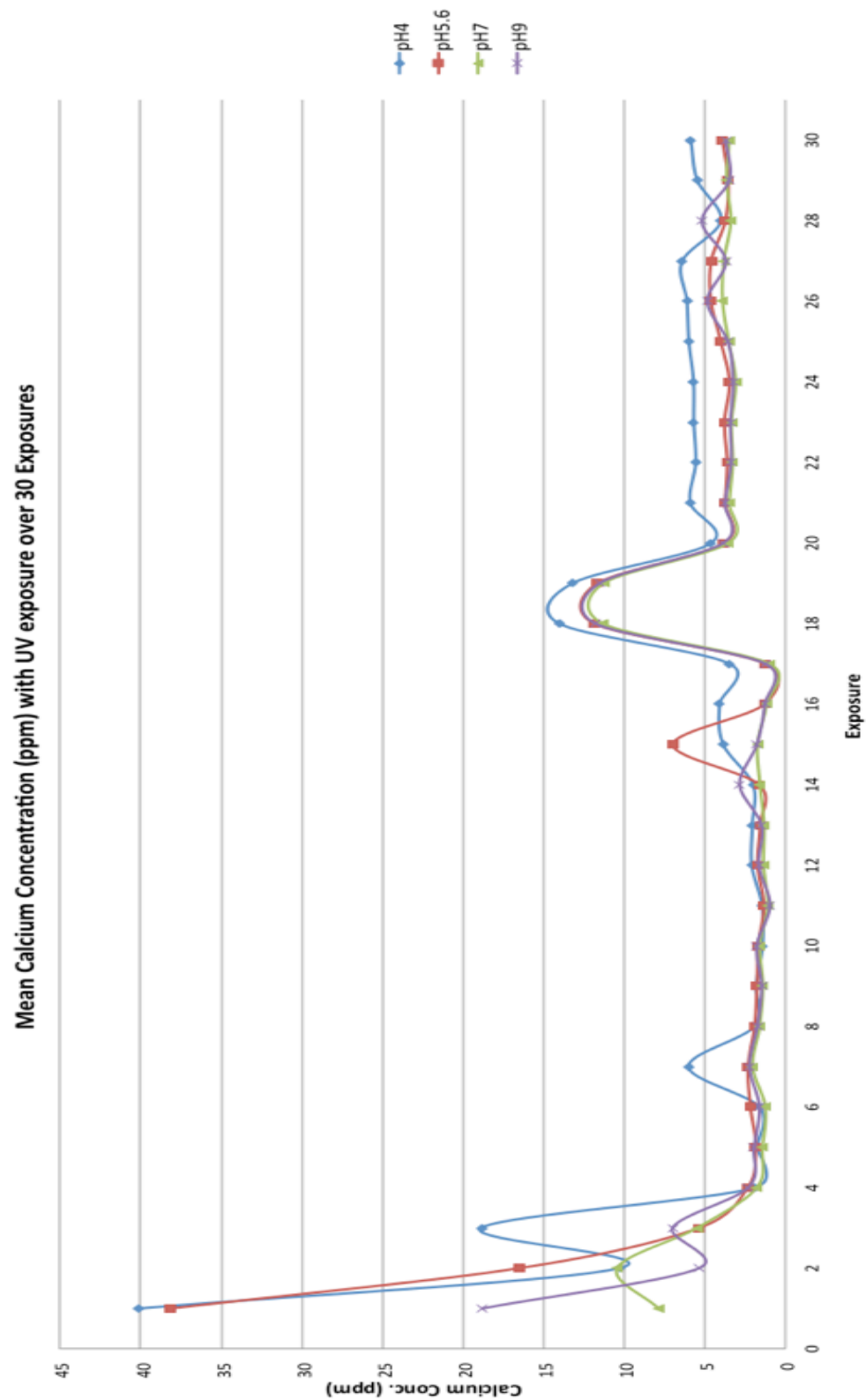


Figure 11: Mean calcium concentrations with UV exposure over 30 exposures

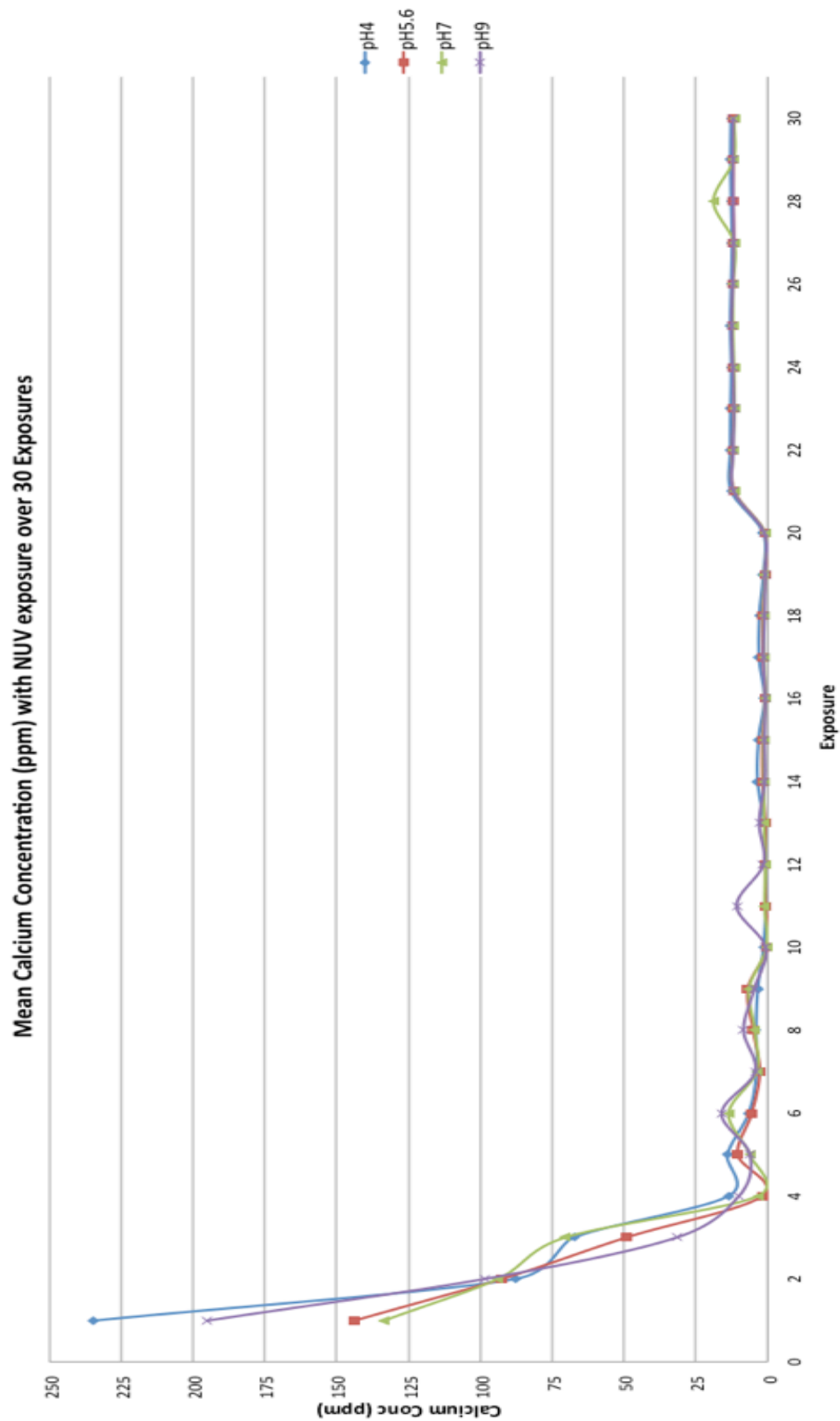


Figure 12: Mean calcium concentrations with no UV exposure over 30 exposures

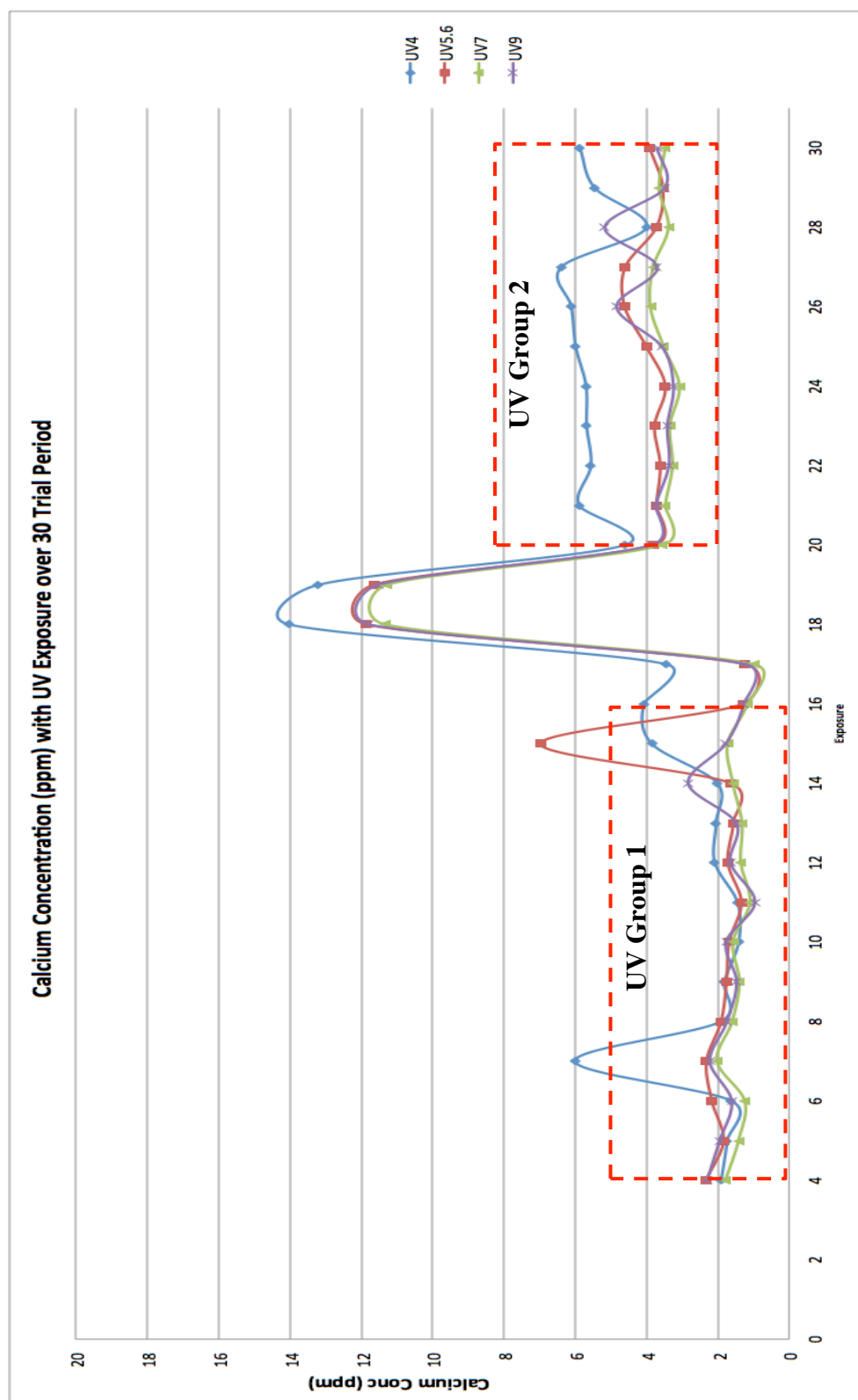


Figure 13: Graph of calcium data identifying UV groups 1 and 2

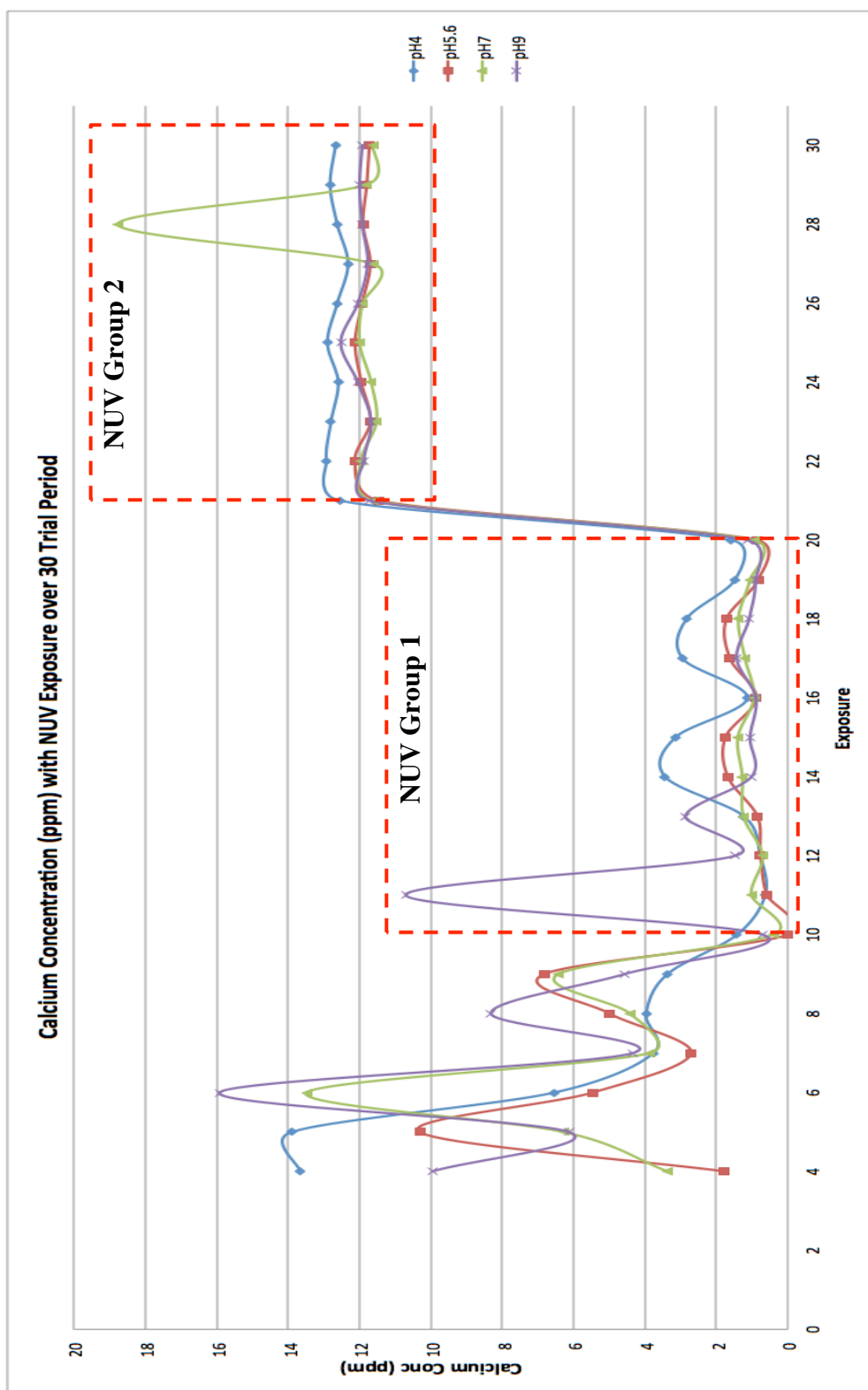


Figure 14: Graph of calcium data identifying NUV groups 1 and 2

DESCRIPTION OF DATA SETS

Data were evaluated by using several statistical tests. A Shapiro-Wilk's test ($p > 0.05$) and an inspection of the skewness and kurtosis measures showed that the log transformed titanium concentration data were approximately normally distributed. A Levene's test of homogeneity ($p > 0.05$) was used to verify the equality of variances in the samples. The same tests were used to evaluate the distribution normality of the calcium concentration data; however, the results showed that the data was not normally distributed, regardless of log transformation and removing outliers. Given this information, log-transformed titanium data was analyzed using parametric statistical tests. Correlation comparisons were made using the Spearman Correlation test, a nonparametric test, when comparing calcium concentration data to titanium concentration data.

ANALYSIS OF EFFECT ON TITANIUM RELEASE BY DIFFERENT pH EXPOSURE

The first objective of this study was to determine if exposure to acidic rain would cause a greater release of titanium dioxide when compared to standard, neutral, and alkaline rains. To evaluate the data for this question, several statistical tests were used for analysis. First, a one-way between subjects ANOVA was conducted comparing the effect of each pH condition on the release of titanium from concrete within UV exposed and UV unexposed groups. Analysis results indicated a significant effect by overall pH conditions on titanium released at the $p < 0.05$ level for UV exposed samples, but no significant effect for UV unexposed samples. To further evaluate the significantly different results, a post hoc comparison with Tukey HSD test ($p < 0.05$) was used, which

indicated no statistically significant difference between pH conditions except between UV 5.6 and UV 9, of which the UV 5.6 condition was significantly less than the UV 9 condition. Lastly, a two-way between subjects ANOVA with interaction was conducted to determine if an interaction effect existed between the UV factor and the pH factor. Results indicated no significant interaction effect between the UV factor and the pH factor. In other words, the difference in titanium concentrations between pH conditions does not vary within the UV exposed and UV unexposed groups, which is additionally supported by the lack of intersecting lines in the means plot (Figure 15).

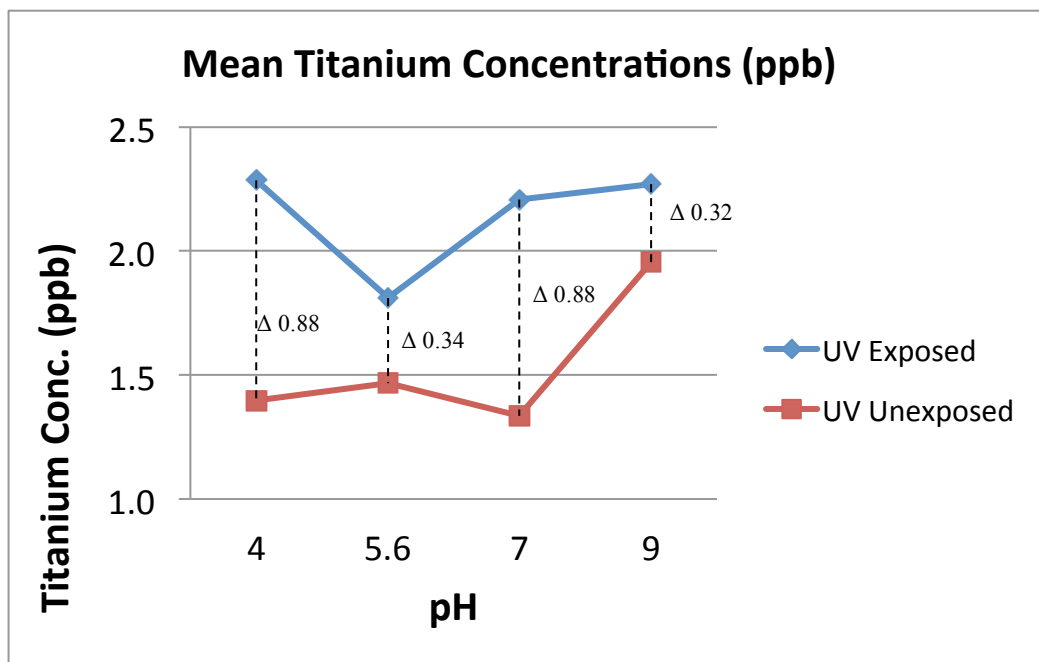


Figure 15: Means plot of mean titanium concentration by UV exposure and pH exposure depicting lack of interaction between pH and UV conditions

ANALYSIS OF EFFECT ON TITANIUM RELEASE BY UV EXPOSURE

The second objective of this research was to determine if UV exposure caused a greater release of titanium. This was determined using several statistical tests. First, a one-way between subjects ANOVA was conducted to compare the effects of UV exposed and UV unexposed conditions on the release of titanium dioxide nanoparticles from concrete. Results indicated a significantly greater difference in mean titanium concentrations between UV exposed data and UV unexposed data, with larger concentrations in the UV exposed group. Post hoc comparisons using the Tukey HSD test ($p < 0.05$) indicated that the mean log concentration for the UV 4 condition was significantly different than the NUV 4 condition and the UV 7 condition was significantly different than the NUV 7 condition. The UV 5.6 condition was not significantly different than the NUV 5.6 condition and the UV 9 condition was not significantly different than the NUV 9 condition. Finally, a two-way between subjects ANOVA was conducted to determine the main effect of the UV condition on the release of titanium. After adjusting for pH, results showed a significant difference in means between the UV exposed and the UV unexposed groups.

Correlation analysis was conducted comparing calcium concentrations with titanium concentrations to provide additional evidence pertaining to the effect of UV exposure on the release of titanium dioxide nanoparticles from concrete. A two-tailed Spearman correlation test ($p < 0.01$) was conducted on the overall calcium and titanium data, as the calcium data was not normally distributed. Inspection of the scatterplot graph (Figure 16) depicts a weak, positive correlation.

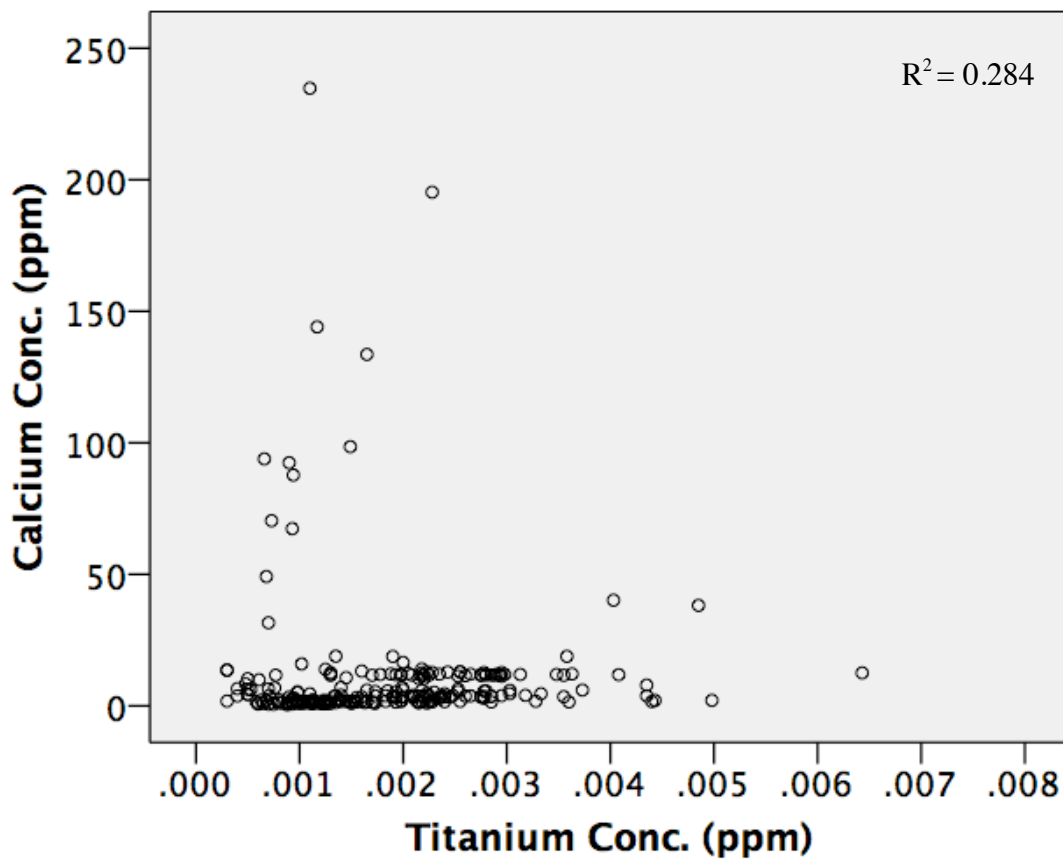


Figure 16: Scatterplot depicting overall weak correlation between calcium and titanium releases

Results of the Spearman Correlation test indicate that there is, in fact, a weak but significant positive relationship between the release of calcium and the release of titanium. This implies, however weakly, that as more calcium is released, more titanium is also released. In consideration of the jumps in the calcium data, the previously defined groups were used to provide a better correlation analysis between the titanium and calcium data. A two-tailed Spearman Correlation test ($p < 0.01$) was conducted comparing the titanium data to the calcium data corresponding to UV Group 1, UV Group 2, NUV Group 1, and NUV Group 2. Visual inspection of the scatterplots for

these groups show no relationship between calcium and titanium release in UV Group 1, UV Group 2, and NUV Group 2. There is a moderately positive relationship in NUV Group 1 (Figure 17). Results from the Spearman Correlation test indicate moderate correlation only in the NUV Group 1 data, suggesting a moderately positive relationship between titanium release and calcium phase specific to this group.

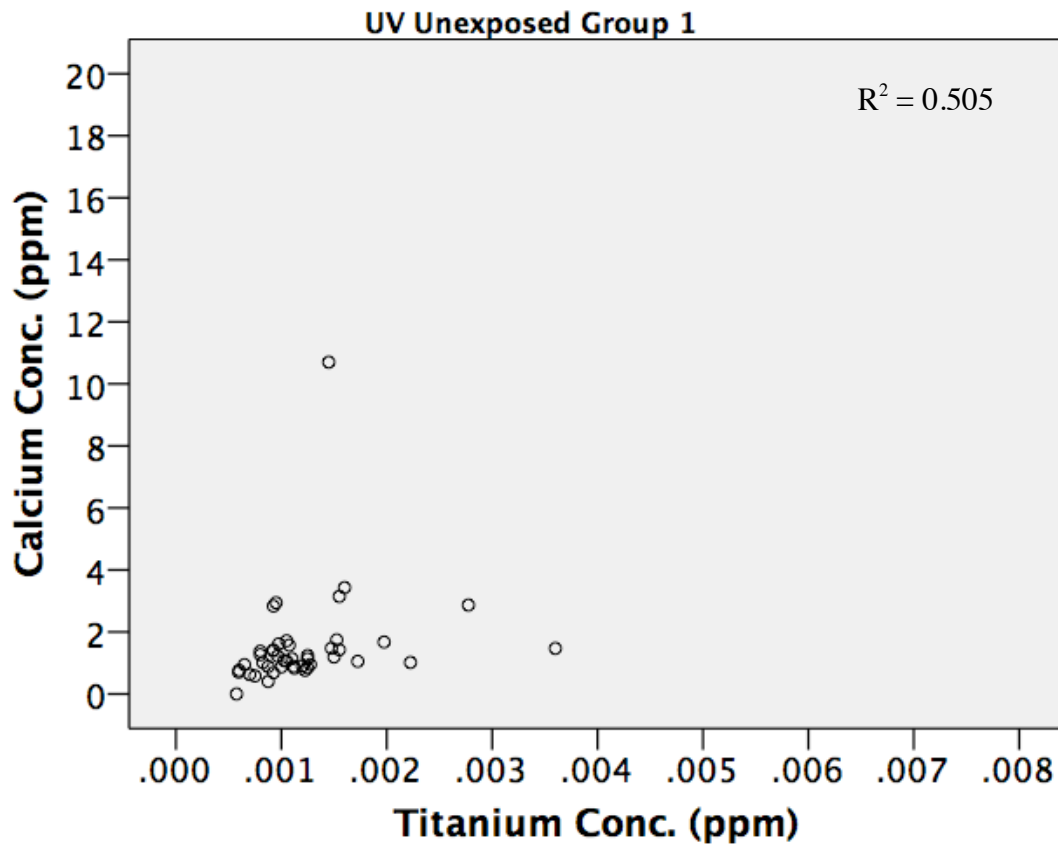


Figure 17: Scatterplot depicting weakly positive relationship between calcium and titanium in UV unexposed Group 1

ANALYSIS OF EFFECT ON TITANIUM RELEASE BY COMBINED UV AND ACIDIC pH SOLUTION EXPOSURE

The third objective of this study was to examine the effect of combined UV exposure and acidic conditions on the release of titanium as compared to UV unexposed, standard, neutral, and alkaline conditions. For this analysis, the post hoc comparisons with the Tukey HSD test ($p < 0.05$) used for the UV analysis indicated that the mean log concentration for the UV 4 condition was significantly different than the NUV 5.6 and NUV 7 conditions; however, was not significantly different than the NUV 9 condition.

CHAPTER 4: DISCUSSION

The purpose of this research was to assess the release of titanium dioxide nanoparticles integrated in concrete as a potential exposure source when weathered under UV and different wet weather conditions. This goal was divided into several questions. The first examined if exposure to different pH solutions, simulating acidic, standard, neutral, and alkaline rains, had an effect on the release of titanium dioxide nanoparticles integrated with concrete. The second examined if exposure to UV radiation had an effect on the release of titanium dioxide nanoparticles integrated with concrete. The third examined if the combined exposure to UV and acidic wet weather conditions in comparison to UV unexposed, standard, neutral, and alkaline wet weather conditions had an effect on the release of titanium dioxide nanoparticles integrated with concrete.

Descriptive results from this research indicate that titanium, as dissolved and particulate titanium measured by ICP-MS, is in fact released when weathered by UV radiation and different wet weather pH conditions. Determining exactly what conditions or combination of conditions caused titanium release was done using statistical comparisons to better understand the data. First, it is important to address the TiO_2 nanoparticle data obtained from the SP-ICP-MS. Results indicated that titanium dioxide nanoparticles were not present in the effluent samples analyzed; however, that does not mean that the titanium released from the test pucks was not nano-titanium dioxide. One limitation of SP-ICP-MS is that it is unable to measure particles less than 20 nm in size. It is possible that some of the particles in the sample solutions were 20 nm or less, but unlikely. Unlikely because the SEM images taken prior to testing indicated that titanium dioxide nanoparticles had agglomerated to form titanium dioxide nanoparticle

agglomerates, or crystals, on the surface of the test pucks. Additionally, SEM images of a test puck after 30 exposures to pH 4 solution, still showed titanium dioxide nanoparticle agglomerates on the surface. It is more likely that the titanium measured by ICP-MS, given the SP-ICP-MS and SEM results, is a mixture of not only dissolved titanium, but also titanium dioxide nanoparticle agglomerates larger than 100 nm in dimension.

Whether these particles are considered nanoparticles depends on the definition of nanoparticles used. As mentioned earlier, different entities have defined nanoparticles differently. The European Union considers agglomerates of nanoparticles to be nanoparticles, regardless of particle size.

When examining the effect of different pH conditions on the release of titanium dioxide nanoparticles from concrete, it was hypothesized that more acidic solutions would cause greater release of nanoparticles. This hypothesis was made based on carbonate chemistry, which can be used to describe how more acidic solutions cause greater dissolution of calcium carbonate, which is a large constituent of concrete. If the concrete was dissolving more readily, it is plausible that the titanium dioxide nanoparticles would similarly dissociate with it. Results from the ANOVA analyses indicate that there was a significant difference between pH conditions in the UV exposed group and no significant difference between pH conditions in the UV unexposed group, suggesting that there is an effect within the pH conditions of the UV group. Additionally, a test for interaction between UV and pH factors indicated that there was no interaction between factors meaning that the differences between pH conditions were indistinguishable. Although there was one significant difference within the UV condition between the pH 5.6 and pH 9 conditions, the pH 5.6 condition was significantly less in

concentration than the pH 9 condition, which does not support the hypothesis. It is not understood why an acidic condition would result in less titanium release than an alkaline condition, but it may be related to the experimental design. Overall, the results indicate that exposure to a certain pH solution may not cause release of titanium dioxide nanoparticles, because there was no difference in titanium release observed between the different pH groups, in both the UV exposed and UV unexposed groups. This outcome may be true; however, given the experimental design, the solutions most likely equilibrated rapidly once exposed to the test puck, subjecting all the test pucks to essentially the same pH solution and resulting in the lack of difference in observed titanium concentrations.

When examining the effect of UV exposure on the release of titanium dioxide nanoparticles integrated with concrete, it was hypothesized that exposure to UV radiation would cause greater release of nanoparticles than no exposure. This hypothesis was made, despite a lack of evidence in literature, presuming that the photoreaction between titanium dioxide and UV photons would cause a negative change in the concrete's integrity, thus releasing any embedded titanium dioxide nanoparticles. Results from the ANOVA test between UV conditions and accounting for the pH factor indicate that titanium is released in significantly greater amounts when exposed to UV radiation when compared to no UV exposure. It is not certain if the release is a direct effect of UV radiation exposure, an effect of secondary variables associated with UV exposure, or a combination of the two. It is possible that the photonic excitation of the titanium dioxide particle may cause release of bonds with concrete. Further investigation would be required to confirm that theory. The increased release may also be associated with the

drying/wetting cycles or heating/cooling cycles inherent to the exposure regimen. Again, additional testing would be required to confirm this theory. The titanium release may also be an effect of the release of calcium as the titanium dioxide nanoparticles are embedded in the concrete. Correlation with calcium data would confirm this theory.

Correlation analysis, intended on providing additional evidence, was a bit more complicated as there were several variables affecting calcium, and potentially titanium, release inherent to the UV and wet weather exposures. As mentioned in the results, the calcium data revealed several issues that were unexpected. First, calcium concentrations were greater in the UV unexposed group compared to the concentrations in the UV exposed group. Inspection of the post-test SEM images indicated that calcium carbonate had re-precipitated on the test pucks in the UV group image, which is an effect of heating/cooling and wetting/drying. Calcium carbonate re-precipitation was not observed in the post-test UV unexposed image. The UV exposed image suggests that any calcium released into the sample solution was then removed from the solution through re-precipitation, resulting in the lower calcium concentrations observed. It also may have been possible that as a result of this re-precipitation of calcium that any titanium dioxide nanoparticles in solution could have re-precipitated as well. This is not supported by the SEM images as individual titanium dioxide nanoparticles or titanium dioxide nanoparticle agglomerates were not observed in the image. It is more likely that any released titanium remained in solution. Second, the large concentration of calcium measured in the first three exposures of both the UV exposed and UV unexposed groups was unlike the rest of the calcium data. It is inferred that these concentrations are the result of powdered, more soluble calcium carbonate left on the surface of the unwashed

test pucks after cutting, and not representative of the dataset. For correlation analysis, these data points were considered outliers and removed. Lastly, the jumps in calcium concentrations in the later exposures indicated another issue making it difficult to do a straight correlation between titanium and calcium. It is very likely that these “jumps” in concentration shows a shift in concrete phase to a more soluble form of calcium compound, which is typical in the concrete curing process (31). As mentioned earlier, groups were made between these concrete phases to provide a better calcium/titanium correlation. The overall correlation, regardless of the issues in the calcium data, comparing titanium and calcium data indicated a weak, positive correlation. Correlation analysis within the prescribed groups (UV Group 1, UV Group 2, NUV Group 1, and NUV Group 2) indicates a moderate, positive correlation between calcium and titanium in UV Group 1 data only. Given these results, it can be suggested that due to lack of correlation in the UV unexposed groups and some correlation in the UV exposed groups that correlating calcium and titanium provides additional evidence supporting the release of titanium dioxide nanoparticles as an effect of UV exposure. More realistically, this correlation analysis does not provide any additional evidence, as the calcium data does not reflect what is expected in accordance with carbonate chemistry and the issues in the calcium data represent confounding variables when making a correlation.

When examining the combined exposure to UV and acidic wet weather conditions (UV 4) in comparison to UV unexposed, standard (NUV 5.6), neutral (NUV 7), and alkaline (NUV 9) wet weather conditions, it was hypothesized that the UV 4 condition would cause greater release of titanium dioxide nanoparticles than the NUV 5.6, NUV 7, or NUV 9 conditions. Results from a post hoc comparison with Tukey HSD test

indicated that differences between the groups were statistically significant, except between the UV 4 condition and the NUV 9 condition. Given that there is no significant difference between the UV 4 and NUV 9 conditions, it does not seem that there is greater release of titanium with combined acidic and UV exposures when compared to combined UV unexposed and less acidic conditions. It would be expected that at lower pH conditions and UV exposure, based on the UV effect findings and carbonate chemistry, that significantly greater concentrations of titanium would be released. It is not the case given these results.

CHAPTER 5: CONCLUSIONS

This research investigated the effects of wet weathering and UV photoactivation on the release of titanium dioxide nanoparticles that have been integrated with concrete for applications in construction materials. The results from this research provide evidence of titanium dioxide nanoparticle's potential as an exposure source when integrated with concrete and weathered under UV and varying wet weather conditions. Additionally, these results may provide reason to further investigate the exposure pathways associated with titanium dioxide nanoparticles and its impact on human health.

This research was conducted by exposing titanium dioxide nanoparticle integrated concrete test pucks to UV radiation in an accelerated UV weathering instrument and then immersing the pucks in pH 4, 5.6, 7, and 9 solutions for an annual exposure equivalent. The solution effluent was then subsequently sampled, analyzing for total titanium and calcium concentrations by mass spectrometry and presence of titanium dioxide nanoparticles by SP-ICP-MS and SEM imaging.

FINDINGS

Initial findings from this research indicate that titanium dioxide nanoparticle agglomerates are released from the titanium dioxide nanoparticle integrated concrete in parts per billion concentrations when weathered under the experimental exposures. Through the use of single particle ICP-MS and scanning electron microscopy, it was concluded that the measured concentrations did not contain any individual titanium dioxide nanoparticles, based on the particle dimension parameter of 100 nm or less. Instead, agglomerates of titanium dioxide nanoparticles were detected, which are still considered nanoparticles because they continue to exhibit the unique properties of the

individual nanoparticle. Thus, the measured titanium concentrations are considered to be representative of the concentration of individual titanium dioxide nanoparticles released from the integrated concrete.

Hypothesis 1: TiO_2 nanoparticles wet weathered in acidic solution will release from concrete substrate in larger concentrations than TiO_2 nanoparticles wet weathered in standard, neutral, and alkaline solutions.

This study found that exposure to more acidic wet weather conditions may not have an effect on the release of titanium dioxide nanoparticles that have been integrated into concrete. Results comparing the difference in means between pH exposures indicate no difference between pH conditions in both UV exposed and UV unexposed samples except for a small significant difference between pH 5.6 and pH 9 in the UV unexposed samples. Although significant, the titanium concentration in the pH 5.6 group is less than the titanium concentration in the pH 9 group. Given these results, the hypothesis that exposure to more acidic solutions causes greater release of titanium dioxide nanoparticles when integrated into concrete cannot be supported.

Hypothesis 2: Photoactivated TiO_2 nanoparticles will release from concrete substrate in larger concentrations than non-photoactivated TiO_2 nanoparticles.

This research found that there was significantly greater release of titanium dioxide nanoparticles in UV exposed samples than in UV unexposed samples. Both the one-way

ANOVA and two-way ANOVA adjusting for the pH condition demonstrated statistically significant difference between UV exposure and no UV exposure with greater concentrations in the UV exposed group, supporting this hypothesis.

Hypothesis 3: UV exposed/Acidic Wet Weathered TiO₂ nanoparticles will release from concrete substrate in larger concentrations than UV unexposed/standard, neutral and alkaline wet weathered TiO₂ nanoparticles.

Findings comparing the difference in extreme conditions (UV 4 conditions vs. NUV 5.6, NUV 7, and NUV 9) indicate that there is significant difference between the UV 4 condition in all pH condition groups except for the NUV 9 condition. Given the lack of significant difference in this group, the hypothesis that the combination of acidic solution and UV exposure causes greater release of titanium dioxide nanoparticles than neutral/alkaline and no UV exposure, cannot be supported.

SIGNIFICANCE

The results of this research indicate that titanium dioxide nanoparticles integrated into concrete are not completely durable and have the potential to release into the environment. Although only detected in ppb amounts, if titanium dioxide nanoparticle integrated concrete use significantly increases, an accumulation of titanium dioxide nanoparticles greater than ppb concentrations may negatively impact the environment and human health. The result that UV exposure increases titanium dioxide nanoparticle release may also impact its future use in concrete, as most concrete is exposed to outdoor conditions. This research is also significant by showing that titanium dioxide

nanoparticles can be an exposure source when integrated with concrete and exposed to UV radiation. This may influence further research into titanium dioxide to include its transportation in the environment, uptake in plant and animal life, exposure route, and potential health effects.

LIMITATIONS

There were several limitations to this research that need to be addressed. First, the accelerated weathering instrument would only support the use of deionized water for spray weathering, so an alternative method of exposing the test pucks to different pH solutions needed to be devised. The experimental design used an immersion method to expose the concrete test pucks, which made it difficult to control the pH of the solution during the exposure. An equilibration experiment was set up prior to the study to determine the amount of time it took a pH 4 solution to reach equilibration when exposed to a concrete test puck; however, this was only done to a UV unexposed test puck and not a UV exposed test puck. Additionally, this experiment was only conducted once to determine the equilibration time. Given this information, it is likely that the solutions, regardless of initial pH, quickly equilibrated, thus effectively nullifying the pH condition of the experiment. This limitation might be corrected for in the future if an accelerated weathering instrument could be altered to accept solutions of different pH for its spray-weathering capability, which is the most realistic simulation of rain weathering. If spray weathering is not possible, immersion testing can still be done. Immersion testing would require the equilibration testing to be done with each pH solution in both UV exposed and UV unexposed conditions, measuring the amount of time to equilibration for each condition. Additionally, during testing, the pH should be measured prior to and after

each wet weather test to ensure that the solutions were the appropriate pH and that they had fully equilibrated.

Second, the make-up of the concrete test pucks was not tested and confirmed, so the composition of the test pucks is not well understood. SEM imaging confirmed the presence of titanium dioxide particles on the surface of the test puck, but it is not understood how the titanium dioxide particles settled during the curing process. This may have influenced the amount of titanium detected in the solution after exposure. This limitation can be prevented in the future by testing the composition of the concrete test pucks and confirming the percent distribution of the titanium dioxide nanoparticles.

Third, the test pucks were not washed prior to testing. This was partly because there was uncertainty how a neutral wash would affect the initial measurements of titanium. The result of not washing the test pucks is evident in the calcium data, where there is an initially large concentration of calcium in the first three exposures (Figures 18-19). Had the test pucks been washed prior to exposure, there may have been a more consistent calcium concentration level measured in the first three exposures.

Fourth, SEM imaging indicated a different morphology on the surface of the UV, pH 4 exposed test puck when compared to the surface of the UV unexposed, pH 4 exposed test puck. The difference was that, with the UV exposure, there was calcium carbonate that had dissolved into solution due to the pH 4 exposure and then re-precipitated onto the test puck as a result of either the wetting/drying exposure variable or the heating/cooling variable associated with the UV exposure. It is believed that this caused release of titanium dioxide nanoparticle agglomerates, but caused lower reported calcium concentrations in the UV exposed samples. This may have caused issues with

the correlations made between the calcium and titanium data or even with the titanium concentrations reported. It is possible that as calcium carbonate re-precipitated, any titanium dioxide nanoparticle agglomerates still adhered to the carbonate material re-precipitated as well.

FUTURE WORK

This research investigated a small part of the exposure pathway as it relates to titanium dioxide nanoparticles that have been integrated with concrete. Given the result that titanium dioxide nanoparticle agglomerates are released when exposed to UV conditions, there is a need to further investigate titanium dioxide nanoparticle's exposure pathway and the mechanisms by which UV radiation impacts its release. The following are recommendations for the further investigation of titanium dioxide nanoparticles integrated with concrete.

(1) Material Related Research

- (a) Investigation into the effects of different pH solutions on the release of titanium dioxide nanoparticle integrated concrete using spray wet weathering instrumentation.
- (b) Investigation into the mechanisms of UV exposure on the release of titanium dioxide nanoparticles from integrated concrete applications.

(2) Public Health Related Research

- (a) Investigation into the transport of titanium dioxide nanoparticle agglomerates into natural waters.
- (b) Investigation into the transformation of titanium dioxide nanoparticle agglomerates in the environment.

- (c) Investigation into the uptake and bioaccumulation of titanium dioxide nanoparticle agglomerates in the aquatic environment.
- (d) Investigation into the health effects related to the consumption of titanium dioxide nanoparticle contaminated water.

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APPENDIX A: SPSS STATISTICAL RESULTS TABLES

Table A-1: Determination of distribution normality in logged titanium data by calculation of z-value from skewness and kurtosis values and respective standard errors.

Titanium Data							
Group	Skewness	Std. Error	Z-Value	Kurtosis	Std. Error	Z-Value	Result
UV 4	-0.403	0.427	-0.94	1.824	0.833	2.18	Not Normal
UV 5.6	-0.026	0.427	-0.06	-0.267	0.833	-0.32	Normal
UV 7	-0.188	0.427	-0.44	0.072	0.833	0.08	Normal
UV 9	-0.406	0.427	-0.95	0.558	0.833	0.66	Normal
NUV 4	-0.433	0.427	-1.01	-0.269	0.833	-0.32	Normal
NUV 5.6	-0.221	0.427	-0.51	0.113	0.833	0.13	Normal
NUV 7	0.055	0.427	0.12	-0.308	0.833	-0.36	Normal
NUV 9	-0.217	0.427	-0.50	-0.632	0.833	-0.75	Normal

Table A-2: Determination of distribution normality in logged titanium data by Shapiro-Wilk test

		Shapiro-Wilk		
		Statistic	df	Sig.
logTiConcpcb	UV4	.954	30	.217
	UV5.6	.980	30	.822
	UV7	.976	30	.700
	UV9	.941	30	.098
	NUV4	.954	30	.217
	NUV5.6	.985	29	.938
	NUV7	.986	30	.946
	NUV9	.953	30	.203

Table A-3: Determination of equality in variances of logged titanium data with Levene's Test for Homogeneity

Test of Homogeneity of Variances

logTiConcppb

Levene Statistic	df1	df2	Sig.
.228	3	235	.877

Table A-4: One-way ANOVA between pH conditions within UV exposure conditions depicting no significant difference in means between pH conditions in UV unexposed titanium data and significant difference in means between pH conditions in UV exposed titanium data

ANOVA

logTiConcppb

UV_NUV		Sum of Squares	df	Mean Square	F	Sig.
NUV	Between Groups	.412	3	.137	1.949	.126
	Within Groups	8.099	115	.070		
	Total	8.511	118			
UV	Between Groups	.317	3	.106	3.272	.024
	Within Groups	3.749	116	.032		
	Total	4.066	119			

Table A-5: Post hoc comparison of titanium UV exposed data between pH exposure conditions

Multiple Comparisons							
Dependent Variable: logTiConcpgb							
Tukey HSD							
UV_NUV	(I) pH	(J) pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
UV	4.0	5.6	.11574	.04641	.066	-.0052	.2367
		7.0	.00828	.04641	.998	-.1127	.1293
		9.0	-.01327	.04641	.992	-.1343	.1077
	5.6	4.0	-.11574	.04641	.066	-.2367	.0052
		7.0	-.10746	.04641	.100	-.2284	.0135
		9.0	-.12902*	.04641	.032	-.2500	-.0080
	7.0	4.0	-.00828	.04641	.998	-.1293	.1127
		5.6	.10746	.04641	.100	-.0135	.2284
		9.0	-.02156	.04641	.967	-.1425	.0994
	9.0	4.0	.01327	.04641	.992	-.1077	.1343
		5.6	.12902*	.04641	.032	.0080	.2500
		7.0	.02156	.04641	.967	-.0994	.1425

*. The mean difference is significant at the 0.05 level.

Table A-6: Two way ANOVA of logged titanium data depicting no interaction between pH and UV conditions

Tests of Between-Subjects Effects					
Dependent Variable: logTiConcpgb					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.803 ^a	7	.400	7.807	.000
Intercept	9.644	1	9.644	188.044	.000
UV_NUV	2.075	1	2.075	40.462	.000
pH	.446	3	.149	2.902	.036
UV_NUV * pH	.279	3	.093	1.812	.146
Error	11.848	231	.051		
Total	24.337	239			
Corrected Total	14.650	238			

a. R Squared = .191 (Adjusted R Squared = .167)

Table A-7: One-Way ANOVA of logged titanium data depicting significant difference in means between UV exposed and UV unexposed conditions

ANOVA

logTiConc_{ppb}

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.074	1	2.074	39.078	.000
Within Groups	12.577	237	.053		
Total	14.650	238			

Table A-8: Post hoc comparison of UV exposed and UV unexposed groups

Multiple Comparisons

Dependent Variable: logTiConcpgb

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
UV4	UV5.6	.11574	.05847	.498	-.0631	.2946
	UV7	.00828	.05847	1.000	-.1706	.1872
	UV9	-.01327	.05847	1.000	-.1921	.1656
	NUV4	.24540*	.05847	.001	.0665	.4243
	NUV5.6	.22312*	.05898	.005	.0427	.4035
	NUV7	.27085*	.05847	.000	.0920	.4497
	NUV9	.11689	.05847	.485	-.0620	.2958
UV5.6	UV4	-.11574	.05847	.498	-.2946	.0631
	UV7	-.10746	.05847	.595	-.2863	.0714
	UV9	-.12902	.05847	.352	-.3079	.0499
	NUV4	.12966	.05847	.345	-.0492	.3085
	NUV5.6	.10737	.05898	.607	-.0730	.2878
	NUV7	.15511	.05847	.143	-.0238	.3340
	NUV9	.00114	.05847	1.000	-.1777	.1800
UV7	UV4	-.00828	.05847	1.000	-.1872	.1706
	UV5.6	.10746	.05847	.595	-.0714	.2863
	UV9	-.02156	.05847	1.000	-.2004	.1573
	NUV4	.23712*	.05847	.002	.0583	.4160
	NUV5.6	.21483*	.05898	.008	.0344	.3952
	NUV7	.26256*	.05847	.000	.0837	.4414
	NUV9	.10860	.05847	.582	-.0703	.2875
UV9	UV4	.01327	.05847	1.000	-.1656	.1921
	UV5.6	.12902	.05847	.352	-.0499	.3079
	UV7	.02156	.05847	1.000	-.1573	.2004
	NUV4	.25868*	.05847	.000	.0798	.4375
	NUV5.6	.23639*	.05898	.002	.0560	.4168
	NUV7	.28412*	.05847	.000	.1053	.4630
	NUV9	.13016	.05847	.340	-.0487	.3090
NUV4	UV4	-.24540*	.05847	.001	-.4243	-.0665
	UV5.6	-.12966	.05847	.345	-.3085	.0492
	UV7	-.23712*	.05847	.002	-.4160	-.0583
	UV9	-.25868*	.05847	.000	-.4375	-.0798
	NUV5.6	-.02229	.05898	1.000	-.2027	.1581
	NUV7	.02544	.05847	1.000	-.1534	.2043
	NUV9	-.12852	.05847	.357	-.3074	.0504
NUV5.6	UV4	-.22312*	.05898	.005	-.4035	-.0427
	UV5.6	-.10737	.05898	.607	-.2878	.0730
	UV7	-.21483*	.05898	.008	-.3952	-.0344
	UV9	-.23639*	.05898	.002	-.4168	-.0560
	NUV4	.02229	.05898	1.000	-.1581	.2027
	NUV7	.04773	.05898	.992	-.1327	.2281
	NUV9	-.10623	.05898	.620	-.2866	.0742
NUV7	UV4	-.27085*	.05847	.000	-.4497	-.0920
	UV5.6	-.15511	.05847	.143	-.3340	.0238
	UV7	-.26256*	.05847	.000	-.4414	-.0837
	UV9	-.28412*	.05847	.000	-.4630	-.1053
	NUV4	-.02544	.05847	1.000	-.2043	.1534
	NUV5.6	-.04773	.05898	.992	-.2281	.1327
	NUV9	-.15396	.05847	.150	-.3328	.0249
NUV9	UV4	-.11689	.05847	.485	-.2958	.0620
	UV5.6	-.00114	.05847	1.000	-.1800	.1777
	UV7	-.10860	.05847	.582	-.2875	.0703
	UV9	-.13016	.05847	.340	-.3090	.0487
	NUV4	.12852	.05847	.357	-.0504	.3074
	NUV5.6	.10623	.05898	.620	-.0742	.2866
	NUV7	.15396	.05847	.150	-.0249	.3328

*. The mean difference is significant at the 0.05 level.

Table A-9: Two-way ANOVA testing the main effects of each condition, depicting significant difference in means between UV exposed and UV unexposed data when adjusted for the pH condition

Tests of Between-Subjects Effects					
Dependent Variable: logTiConcppb					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.524 ^a	4	.631	12.177	.000
Intercept	9.629	1	9.629	185.806	.000
UV_NUV	2.083	1	2.083	40.192	.000
pH	.450	3	.150	2.897	.036
Error	12.126	234	.052		
Total	24.337	239			
Corrected Total	14.650	238			

a. R Squared = .172 (Adjusted R Squared = .158)

Table A-10: Overall Spearman correlation test of calcium and titanium data

Correlations			Calcium	Titanium
Spearman's rho	Calcium	Correlation Coefficient	1.000	.284**
		Sig. (2-tailed)	.	.000
		N	239	238
	Titanium	Correlation Coefficient	.284**	1.000
		Sig. (2-tailed)	.000	.
		N	238	239

** . Correlation is significant at the 0.01 level (2-tailed).

Table A-11: Spearman correlation results of comparisons between titanium and calcium data within identified calcium groups

Correlations				Calcium	Titanium
Group					
1	Spearman's rho	Calcium	Correlation Coefficient	1.000	-.051
			Sig. (2-tailed)	.	.719
			N	52	52
		Titanium	Correlation Coefficient	-.051	1.000
			Sig. (2-tailed)	.719	.
			N	52	52
2	Spearman's rho	Calcium	Correlation Coefficient	1.000	.153
			Sig. (2-tailed)	.	.300
			N	48	48
		Titanium	Correlation Coefficient	.153	1.000
			Sig. (2-tailed)	.300	.
			N	48	48
3	Spearman's rho	Calcium	Correlation Coefficient	1.000	.505**
			Sig. (2-tailed)	.	.000
			N	44	44
		Titanium	Correlation Coefficient	.505**	1.000
			Sig. (2-tailed)	.000	.
			N	44	44
4	Spearman's rho	Calcium	Correlation Coefficient	1.000	.143
			Sig. (2-tailed)	.	.379
			N	40	40
		Titanium	Correlation Coefficient	.143	1.000
			Sig. (2-tailed)	.379	.
			N	40	40

** . Correlation is significant at the 0.01 level (2-tailed).

APPENDIX B: ADDITIONAL GRAPHS

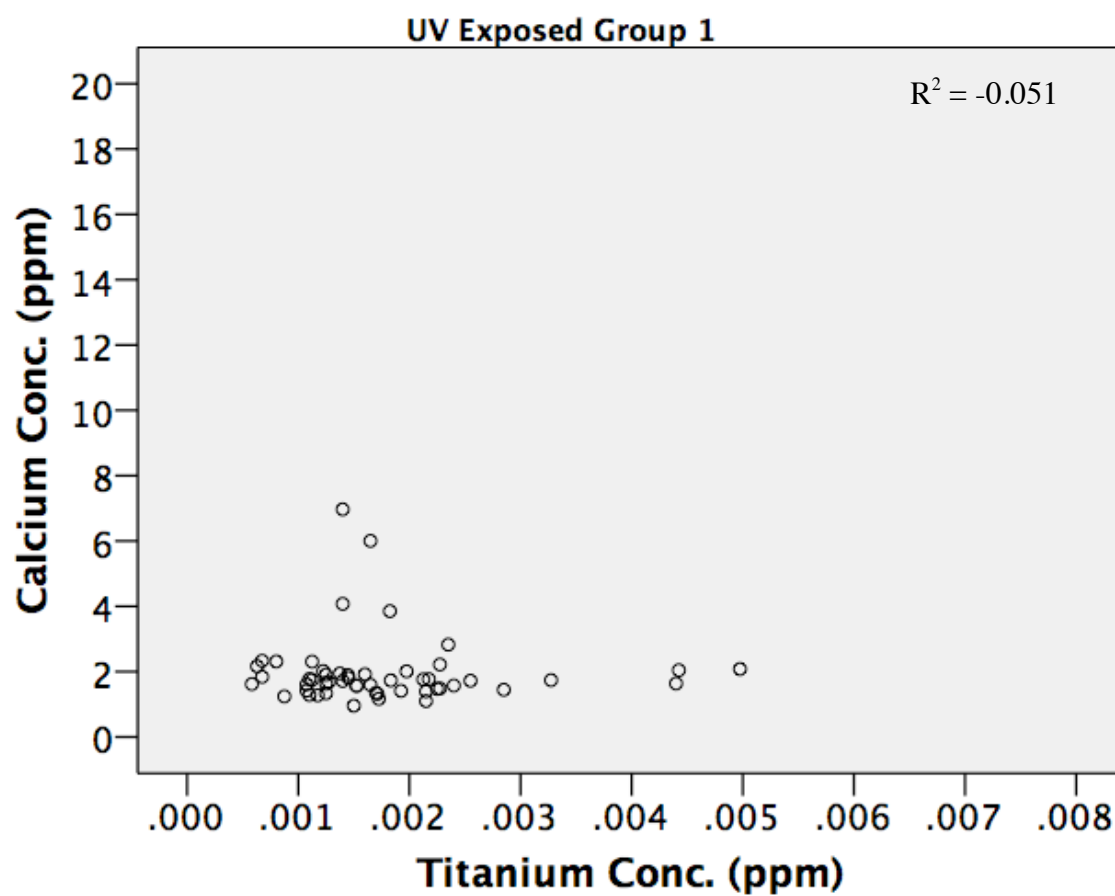


Figure B-1: Scatterplot correlation graph of calcium and titanium data in UV exposed Group 1

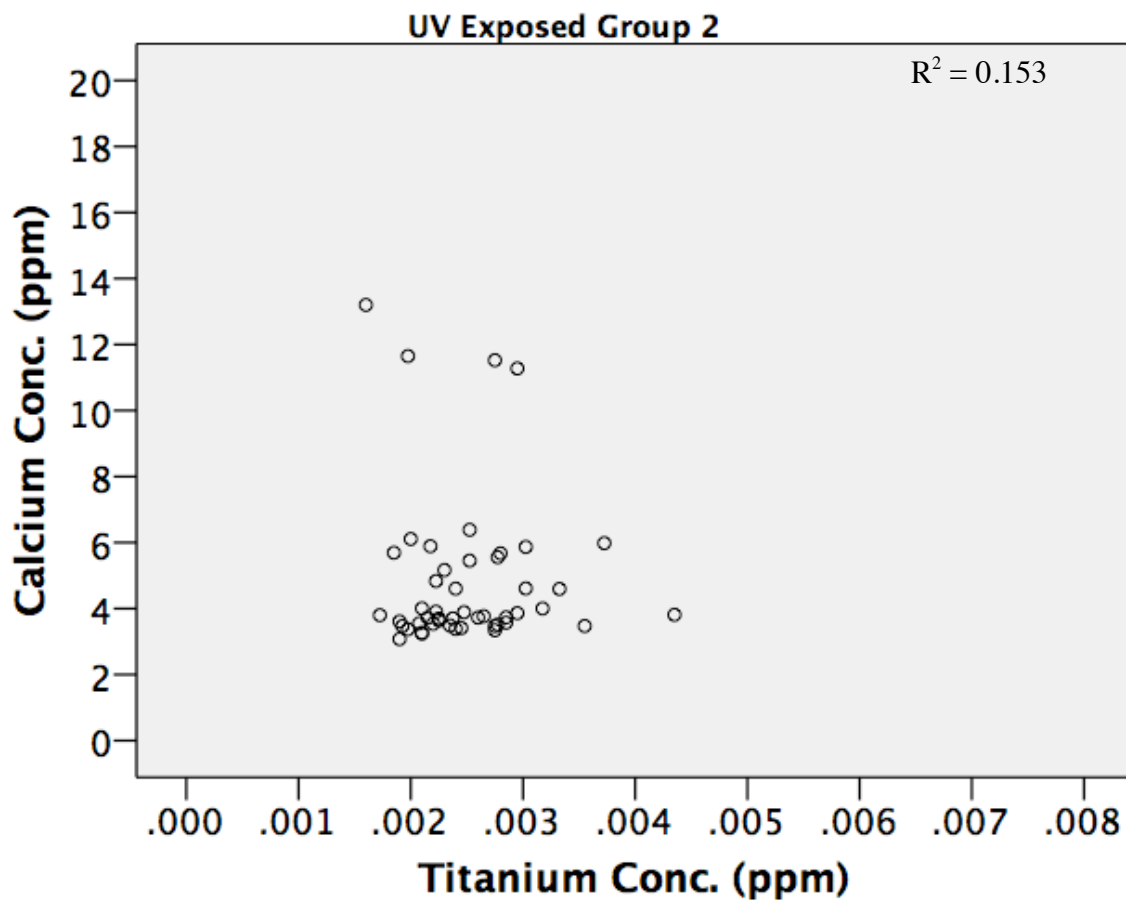


Figure B-2: Scatterplot correlation graph of calcium and titanium data in UV exposed Group 2

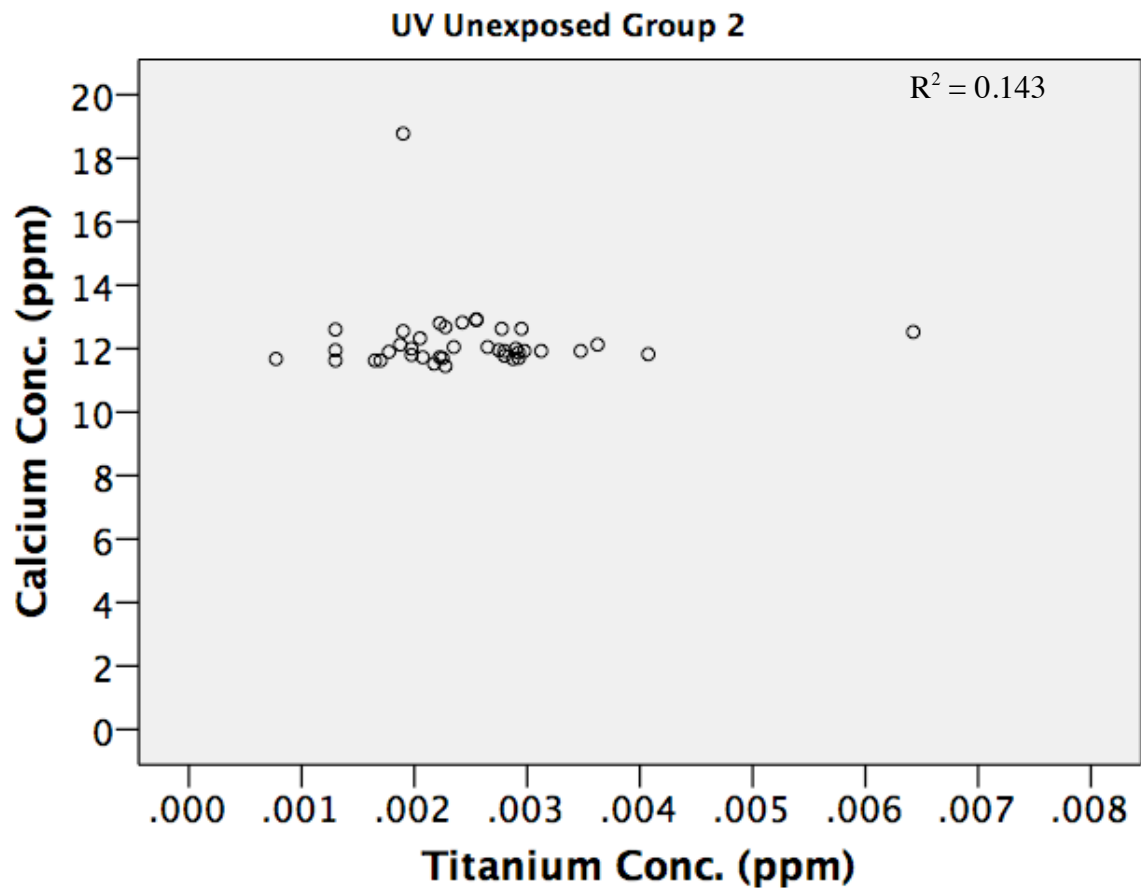


Figure B-3: Scatterplot correlation graph of calcium and titanium data in UV unexposed group 2